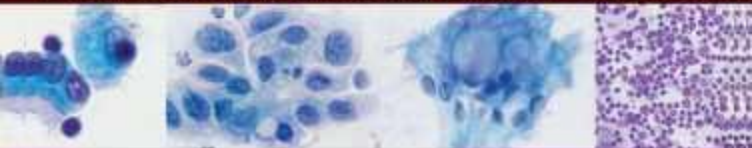


Cytology

Diagnostic Principles and Clinical Correlates



Edmund S. Cibas
Barbara S. Ducatman

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CYTOLOGY: DIAGNOSTIC PRINCIPLES AND
CLINICAL CORRELATES

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*Dedicated to
Todd Bryant Stewart
and
Alan M. Ducatman*

PREFACE TO THIRD EDITION

We hope this book will serve as a useful guide both for the pathologist in practice and for the trainee—resident or fellow—who is looking to obtain expertise in this subspecialty.

It has been 5 years since the publication of the second edition of *Cytology: Diagnostic Principles and Clinical Correlates*. Since then, cytology has continued to grow and evolve as a subspecialty devoted to the diagnosis of cellular tissue obtained by minimally invasive methods (scraping, brushing, aspiration, etc.), and thus the need for this updated edition. But we have retained many of the qualities of the prior editions. As did the first two, this edition aims to be concise yet comprehensive. We have emphasized brevity and clarity. The text is grounded firmly in an understanding of surgical pathology and current diagnostic terminology. Where relevant, we have illustrated the value of established ancillary studies (e.g., flow cytometry and immunohistochemistry) as well as evolving techniques such as cytogenetics, which can be helpful in the diagnosis of certain lymphomas, soft tissue tumors, renal neoplasms, and mesothelioma.

Although the book is multi-authored, the chapters follow a similar format: indications, sample collection and preparation methods, recommended terminology for reporting results, accuracy (including common pitfalls

that lead to false-negative and false-positive diagnoses), a description of normal elements, and finally, a how-to guide for the diagnosis of benign and malignant lesions, with an emphasis on differential diagnosis. We have retained the bulleted “capsule summaries,” particularly for summarizing cytomorphologic features and differential diagnoses. We have continued to emphasize clinical correlation (hence the title). For example, Chapter 1 includes the recently revised algorithms of the American Society for Colposcopy and Cervical Pathology for managing women with abnormal cervical cytologic diagnoses. Good cytologists are those who understand the clinical implications of their interpretations.

Once again, we hope we have succeeded in conveying the beauty, strength, and challenge of cytology. With this book we have tried to take some of the mystery out of cytology. But mysteries remain; their solutions still obscure. If this text inspires the reader to explore and even solve some of them, we will consider ourselves doubly rewarded.

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2008

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We are indebted to many members of the staff of the Brigham and Women's Hospital and West Virginia University School of Medicine and Hospital—the cytotechnologists, cytopathologists, and trainees—who inspire us with their devotion to cytopathology and who continue to challenge us. In particular, we wish to acknowledge Dorothy Nappi, CT (ASCP), and Grace Goffi, CT (ASCP) (IAC), who have helped us train so many pathology residents and fellows over the years. Without their help we would not have our extraordinary collections of cytology teaching cases from which so many of the images in this book are derived.

Finally, to our friends, families, and loved ones, especially Todd Stewart and Alan Ducatman, who tolerated the long evening and weekend hours that deprived them (temporarily!) of a large share of our time. This book would not exist without their love and strength.

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Cervical and Vaginal Cytology

EDMUND S. CIBAS

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ENDOMETRIAL CELLS IN WOMEN OLDER THAN 40 YEARS OF AGE

The 20th century witnessed a remarkable decline in the mortality from cervical cancer in many developed countries. This achievement is directly attributable to the implementation of the Papanicolaou (Pap) test. In the 1930s, before Pap screening was introduced, cervical

cancer was the most common cause of cancer deaths in women in the United States.¹ Today, it is not even one of the top ten.²

The incidence of cervical cancer in the United States is approximately 11,000 cases, with 3670 deaths.²

Worldwide, however, the cervical cancer incidence (over 500,000 cases annually) and mortality rates (288,000 deaths per year) are second only to those for breast cancer.³ Screening programs, unfortunately, are rudimentary or nonexistent in many parts of the world. Fewer than 5% of women in developing countries have ever had a Pap test.⁴ In contrast, 89% of women in the United States report having had a Pap test in the preceding 3 years.

THE HISTORY OF THE PAP TEST

The Pap test is considered by many to be the most cost-effective cancer reduction program ever devised.¹ Credit for its conception and development goes to George N. Papanicolaou, an anatomist and Greek immigrant to the United States. In 1928 he reported that malignant cells from the cervix can be identified in vaginal smears.⁵ Later, in collaboration with the gynecologist Herbert Traut, who provided him with a large number of clinical samples, Papanicolaou published detailed descriptions of preinvasive cervical lesions.^{6,7} Pathologists and physicians initially greeted this technique with skepticism, but by the late 1940s Papanicolaou's observations had been confirmed by others. The Canadian gynecologist J. Ernest Ayre suggested taking samples directly from the cervix with a wooden spatula rather than from the vagina with a pipette as originally described by Papanicolaou.⁸ Eventually, cytologic smears were embraced as an ideal screening test for preinvasive lesions, which, if treated, would be prevented from developing into invasive cancer.

The first cervical cancer screening clinics were established in the 1940s.⁹ The Pap test was never evaluated in a controlled, prospective study, but several pieces of evidence link it to the prevention of cervical cancer. First, the mortality rate from cervical cancer fell dramatically after screening was introduced, by 72% in British Columbia¹⁰ and 70% in Kentucky.¹¹ Second, there was a direct correlation between the intensity of screening and the decrease in mortality. Among Scandinavian countries, the death rate fell by 80% in Iceland, where screening was greatest; in Norway, where screening was lowest, the death rate fell by only 10%.¹² A similar correlation was observed in high and low screening regions of Scotland¹³ and Canada.¹⁴ In the United States, the decrease in deaths from cervical cancer was proportional to the screening rates in various states.¹⁵ Finally, women who do not develop invasive cancer are more likely to have had a Pap test than women with cancer. In a Canadian study, the relative risk for women who had not had a Pap test for 5 years was 2.7,¹⁶ and screening history was a highly significant risk factor independent of other factors such as age, income, education, sexual history, and smoking. In Denmark, a woman's risk of developing cervical cancer decreased in proportion to the number

of negative smears she had had, by 48% with just one negative smear, 69% with two to four negative smears, and 100% with five or more smears.¹⁷

Screening guidelines differ around the world. Even in the United States, the recommendations of different organizations vary in some of their details.¹⁸⁻²⁰ The American Cancer Society (ACS) recommends the following:

- Cervical cancer screening should begin approximately 3 years after a woman begins having vaginal intercourse, but no later than 21 years of age.
- Until age 30, cervical screening should be carried out every year with conventional Pap tests or every 2 years using liquid-based Pap tests.
- At or after age 30, a woman who has had three normal test results in a row may be screened every 2 to 3 years with a Pap test (smear or liquid-based) or every 3 years with a Pap plus human papillomavirus (HPV) test.
- A woman 70 years of age and older who has had three or more normal Pap test results and no abnormal results in the previous 10 years may choose to stop cervical cancer screening.
- A woman who has had a total hysterectomy may choose to stop cervical cancer screening. (Exceptions are women with a history of CIN 2,3, cervical cancer, or in utero diethylstilbestrol [DES] exposure.)

Women with a history of cervical cancer, in utero DES exposure, and who are immunocompromised (organ transplantation, chemotherapy, chronic corticosteroid treatment, or positive for human immunodeficiency virus [HIV]) may benefit from more frequent screening.¹⁹ Adherence to these guidelines is critical for cervical cancer prevention. In the United States, more than 50% of women who develop cervical cancer have not had a Pap test in the 3 years before their cancer diagnosis.²¹

The recent development of two prophylactic HPV vaccines provides a new opportunity for cervical cancer prevention.³ Both vaccines consist of empty protein shells called virus-like particles that are made up of the major HPV capsid protein L1. They contain no DNA and are not infectious. One of the vaccines, Gardasil (Merck & Co., Inc.), is a quadrivalent vaccine against HPV types 6, 11, 16, and 18. The other is the bivalent vaccine Cervarix (GlaxoSmithKline) that protects against HPV 16 and 18. They have shown extraordinary efficacy in preventing type-specific histologic CIN 2,3 lesions, with no difference in serious adverse effects compared to placebo.²² The vaccines are administered in three doses to females ages 9 to 26 years before the initiation of sexual activity. Continued Pap screening will remain important for many decades, however, because these vaccines do not protect against 30% of cervical cancers (i.e., those not related to HPV 16 or 18); the duration of protection is unknown; they are not effective in treating prevalent HPV infections; and the cost of the vaccines might limit their use in some populations.³

As seen in the aforementioned ACS recommendations, the combination of a Pap test plus HPV test is included as an option for screening women 30 years of age or older. The rationale is to combine the superior sensitivity of HPV testing with the superior specificity of the Pap test. This recommendation is controversial because it increases screening costs. Moreover, questions remain regarding the ideal management of women with discrepant results (e.g., HPV test positive and Pap negative). The search for the best screening algorithm will undoubtedly continue, particularly as molecular diagnostic methods become more readily available.

SAMPLING AND PREPARATION METHODS

To obtain an ideal Pap specimen, the following guidelines have been established by the Clinical and Laboratory Standards Institute.²³

Patient instructions:

- Schedule the examination 2 weeks after the first day of the last menstrual period. (It is preferable to avoid examination during menses because blood may obscure significant findings.)
- Do not use vaginal medication, vaginal contraceptives, or douches for 48 hours before the appointment.
- Intercourse is not recommended the night before the appointment.

Specimen collection:

- Specimens should be obtained after a nonlubricated speculum (moistened only with warm water if needed) is inserted.
- Excess mucus or other discharge should be removed gently with ring forceps holding a folded gauze pad.
- The sample should be obtained before the application of acetic acid or Lugol iodine.
- An optimal sample includes cells from the ectocervix and endocervix.

Recent studies have challenged the prohibition against a lubricated speculum and suggest that water-based lubricants may be acceptable.²⁴

Conventional Smears

Conventional smears are often obtained using the combination of a spatula and brush. The spatula is used first. Although a wooden or plastic spatula is acceptable, the

plastic spatula is recommended because wooden fibers trap diagnostic material. The spatula is rotated at least 360 degrees. The sample can be smeared on one half of a slide and spray fixed (the other half should be covered to avoid coating it with fixative before the endocervical sample is applied). Alternatively, one may set aside the spatula sample momentarily while the endocervical brush sample is obtained.

After the brush is inserted in the endocervical canal, some bristles should still be visible. If inserted too far, there may be inadvertent sampling of the lower uterine segment (LUS), which causes diagnostic difficulties because its epithelium resembles a high-grade squamous intraepithelial lesion (HSIL) and adenocarcinoma in situ (AIS). The brush should be rotated gently only one-quarter turn. A larger rotation is unnecessary because the circumferential bristles are in contact with the entire surface the moment the brush is inserted.

The spatula sample, if not already applied and fixed, should be applied to the slide, then the brush sample rolled over the slide, followed by immediate fixation. The two samples can be placed in quick succession on two separate halves of the slide, or the endocervical sample can be rolled directly over the spatula sample, both covering the entire slide. Immediate fixation (within seconds) is critical to prevent air-drying artifact, which distorts the cells and hinders interpretation.

The broomlike brush (“broom”) has a flat array of plastic strips contoured to conform to the cervix, with longer strips in the middle. This design allows simultaneous sampling of the endocervix and ectocervix. The long middle strips are inserted into the os until the shorter outer strips bend against the ectocervix. The broom is rotated three to five times. To transfer the material, each side of the broom is stroked once across the slide in a painting motion.

The cotton swab moistened with saline is no longer recommended because its fibers trap cells, reducing the efficiency of cell transfer onto slides.

There are two options for smear fixation. Coating fixatives contain alcohol and polyethylene glycol and are applied by pump sprays, by droppers from dropper bottles, or by pouring from an individual envelope included as part of a slide preparation kit. Alternatively, the smear can be immersed directly into a container filled with 95% ethanol.

Samples for liquid-based cytology (LBC) are obtained as described except that, instead of smearing the cells on a slide, the collection device is rinsed in a vial containing a liquid fixative. In the United States, the LBC Pap test is more common than the smear.

Liquid-Based Cytology

An important landmark in the history of the Pap test occurred in 1996 when the U.S. Food and Drug

Administration (FDA) approved the **ThinPrep™** (Hologic, Marlborough, Mass.) as an alternative to the conventional cervicovaginal smear. This was followed 3 years later by approval of the AutoCyte Prep™ (now known as **SurePath™**; BD TriPath, Burlington, NC). The newest LBC is the **MonoPrep™** (MonoGen, Inc., Lincolnshire, Ill.), which was approved in 2006. LBCs were an important step in the development of automated Pap screening devices—an improved preparation was needed to minimize cell overlap so that automated screeners would perform better in identifying abnormal cells. But LBC performed so well in clinical trials against conventional smears that it found a market independent of automated screening. Although there are exceptions,²⁵ the great majority of peer-reviewed studies, some of them detailed in this chapter, show an increased detection of low-grade squamous intraepithelial lesions (LSILs) or HSILs with LBC.²⁶ The debate over increased disease detection with LBC continues, however, and the studies comparing LBC to smears have come under criticism for allegedly sacrificing methodologic purity in their design.²⁶ Nevertheless, LBC offers several clear advantages over conventional smears: the opportunity to prepare duplicate slides and even cell block preparations from the residual sample;^{27,28} the option of “out-of-vial” aliquoting for HPV, chlamydia, and gonorrhea testing; an improved substrate for automated screening devices; and a thinner cell preparation that most pathologists and cytotechnologists find less tiring to review than smears.

ThinPrep Pap Test

The practitioner obtains the ThinPrep Pap sample with either a broom-type device or a plastic spatula/endocervical brush combination. The sampling device is swirled or rinsed in a methanol-based preservative solution (PreservCyt) for transport to the cytology laboratory and then discarded. Red blood cells are lysed by the transport medium. The vials are placed one at a time on the ThinPrep 2000 instrument. The entire procedure (Fig. 1.1A) takes about 70 seconds per slide and results in a thin deposit of cells in a circle 20 mm in diameter (contrast with cytospin: diameter = 6 mm). A batch-processing version (the ThinPrep 3000) is also available. It uses the same consumables (filters and solutions) but allows automated processing of 80 samples at one time. In most cases, only a fraction of the sample is used to prepare the slide used for diagnosis. If needed, the residual sample is available for additional ThinPrep slide preparation, cell block preparation, or molecular diagnostic testing (e.g., high risk HPV, chlamydia, gonorrhea).

A multicenter, split-sample study found that the ThinPrep detected 18% more cases of LSILs and more serious lesions as compared to conventional smears, with no significant difference in the detection of organisms.²⁹ A number of studies have shown significant

increases in HSIL detection after the implementation of the ThinPrep.^{30–35}

The ThinPrep is equivalent to the conventional smear in the detection of endocervical AIS.³⁶ Data also show comparable results between ThinPrep slides and conventional smears for the detection of endometrial pathology.³⁷

The ThinPrep collection vial has been approved by the FDA for direct testing for HPV, which is particularly useful for managing women whose Pap tests show atypical squamous cells (ASC).^{38,39}

SurePath Pap Test

TriPath Imaging (acquired by Becton Dickinson in 2006) developed the SurePath Pap test (formerly AutoCyte Prep and CytoRich) for samples collected in an ethanol-based transport medium. The process is shown in Figure 1.1B. In contrast to the ThinPrep and MonoPrep methods, the practitioner snips off the tip of the collection device and includes it in the sample vial. The equipment to prepare slides includes a Hettich centrifuge and a PrepStain robotic sample processor with computer and monitor. The PrepMate™ is an optional accessory that automates mixing the sample and dispensing it onto the density reagent. Red blood cells and some leukocytes are eliminated by density centrifugation. In addition to preparing an evenly distributed deposit of cells in a circle 13 mm in diameter, the method incorporates a final staining step that discretely stains each individual slide.

A multicenter, split-sample clinical trial showed a 7.2% increase in the detection of LSILs and more serious lesions and a significant decrease in the percentage of unsatisfactory specimens.⁴⁰

MonoPrep Pap Test

The practitioner obtains the MonoPrep sample with standard collection devices that are swirled or rinsed in a preservative-filled collection vial, after which the sampling device is discarded. As with the ThinPrep, red blood cells are lysed by the transport medium. The vials are delivered to the laboratory where slides are prepared using the MonoPrep Processor, a fully automated, batch-processing instrument capable of processing 40 samples per hour, with a throughput capacity of 324 samples per 8-hour run. The process is shown in Figure 1.1C. In a split-sample clinical trial similar in design to the ThinPrep and SurePath trials, slides prepared by the MonoPrep method showed a 26% increase in the detection of LSILs and more serious lesions, with no significant difference in relative specificity.⁴¹ MonoPrep provided a significant reduction in unsatisfactory slides, and there was no difference in the presentation of endocervical or transformation zone component or the detection of benign conditions.

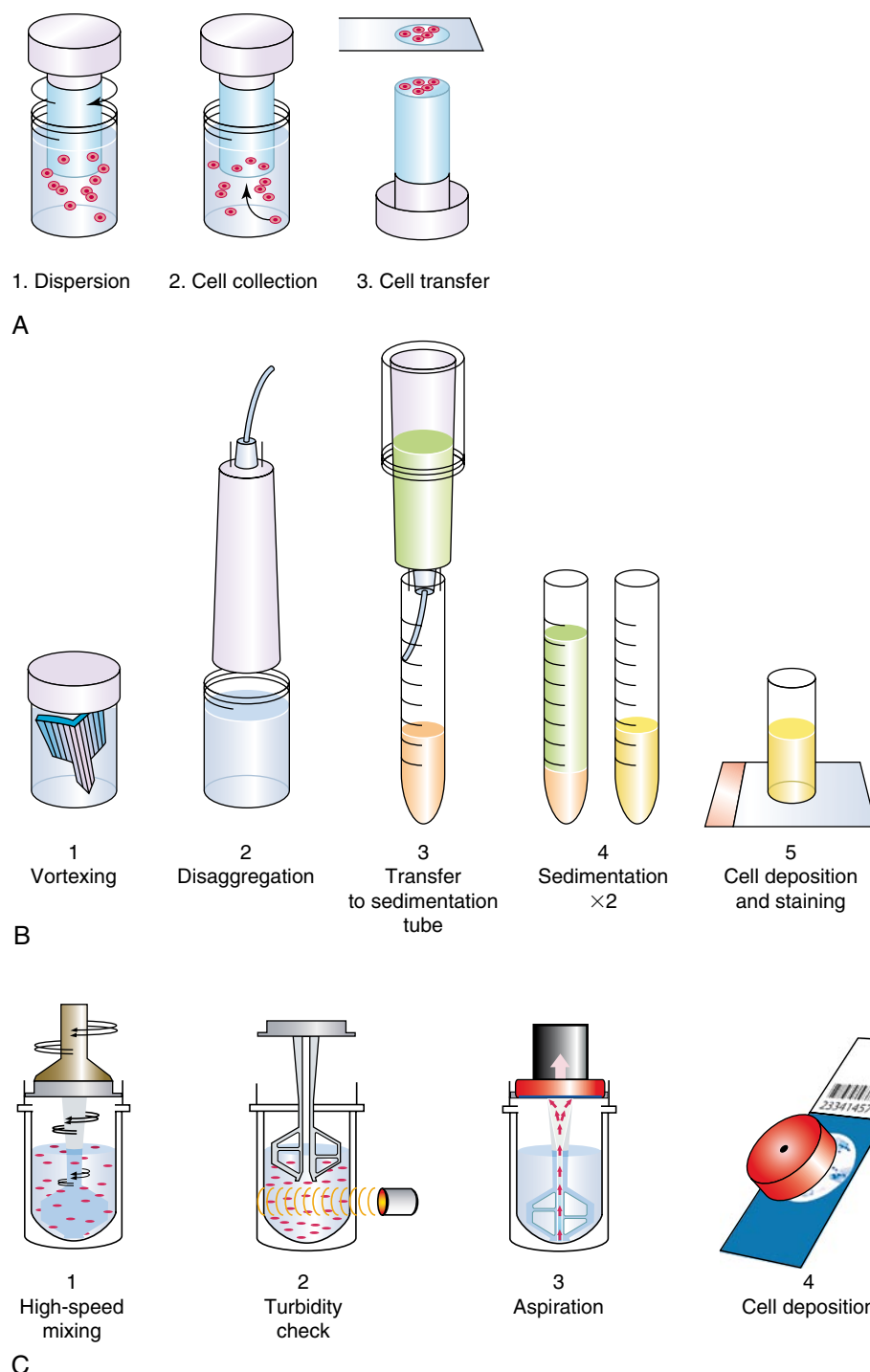


Figure 1.1 Liquid-based slide preparation methods. A, ThinPrep method: 1. The sample vial sits on a stage and a hollow plastic cylinder with a 20-mm diameter polycarbonate filter bonded to its lower surface is inserted into the vial. A rotor spins the cylinder for a few seconds, homogeneously dispersing the cells. 2. A vacuum is applied to the cylinder, trapping cells on the filter. The instrument monitors cell density. 3. With continued application of vacuum, the cylinder (with cells attached to the filter) is inverted 180 degrees, and the filter is pressed against a glass slide. The slide is immediately dropped into an alcohol bath. B, SurePath method: 1. The sample is quickly vortexed. 2. A proprietary device, the Cytro™, disaggregates large clusters by syringing the sample through a small orifice. 3. The sample is poured into a centrifuge tube filled with a density gradient reagent. 4. Sedimentation is performed in a centrifuge. A pellet is obtained and resuspended, and the sedimentation is repeated. 5. The tubes are transferred to the PrepStain instrument, where a robotic arm transfers the fluid into a cylinder. Cells settle by gravity onto a cationic polyelectrolyte-coated slide. The same robotic arm also dispenses sequential stains to individual cylinders. C, MonoPrep method: 1. An integrated stirrer mixes the specimen briefly to disperse mucus and aggregates. 2. The specimen is aspirated into the hollow stirrer and dual-flow technology captures a representative sample on a frit-backed filter. 3. The filter is pressed against the slide to transfer the cells onto a 20-mm diameter circular area. 4. After cell transfer, the instrument applies a premeasured amount of alcohol fixative directly onto the slide.

AUTOMATED SCREENING

Historical Overview

Automated cytology screening devices have been under development since the 1950s. The first computerized screening system was developed in the United States by Airborne Instruments Inc., and was called the Cytoanalyzer.⁴² In preclinical trials it did not perform as well as expected and the project was discontinued. The difficulty of the task was soon appreciated, especially the inherent problems with analyzing smears prepared in the conventional manner. Despite setbacks, research into cervical cytology screening continued, especially in Europe and Japan, throughout the 1970s and 1980s, with the development of the Quantimet,⁴³ BIOPEPR,⁴⁴ CERVIFIP,⁴⁵ CYBEST,⁴⁶ DIASCANNER,^{47,48} FAZYTAN,⁴⁹ and LEYTAS.⁵⁰ Some of these instruments are now in museums, but others have served as prototypes for systems that are commercially available or still under development.

Although European investigators largely lost interest in cytology automation in the 1990s,⁵¹ researchers in the United States and Canada, having established private enterprises supported by venture capital, retained their enthusiasm. Foremost in the field have been AutoCyte (formerly Roche Image Analysis Systems), Cytyc, Neopath, and Neuromedical Systems. An important three-way merger took place in 1999, when AutoCyte, after purchasing the intellectual property of Neuromedical Systems, merged with Neopath to form a new company called TriPath Imaging. In 2007, Cytyc Corporation, developer of the ThinPrep Pap Test and ThinPrep Imaging System, merged with Hologic Inc., and became a wholly-owned subsidiary of Hologic.

In 1998, the FDA approved the AutoPap System (now called the FocalPoint Slide Profiler™; BD TriPath Imaging, Burlington, NC) as a primary screener for cervicovaginal smears, followed by approval in 2002 for use with SurePath slides. In 2003, the FDA approved the ThinPrep Imaging System™ as a primary screener for ThinPrep Pap slides. Thus, these two automated screening devices are designed for different preparation methods. Although both rely on image analysis technology, there are also fundamental differences in the way they integrate into the workflow of the laboratory. Neither is approved for use for nongynecologic cytology specimens.

FocalPoint Slide Profiler

The FocalPoint Slide Profiler (FPSP) is a self-contained instrument that classifies Pap slides without human intervention (Fig. 1.2A). It uses algorithms to measure cellular features like nuclear size, integrated optical density, nuclear to cytoplasmic ratio, and nuclear contour—

morphologic features that pathologist Stanley Patten established using planimetry and ocular micrometry for the diagnosis of squamous and glandular lesions.⁵²

AutoPap, the predecessor of FPSP, was originally intended as a primary screening device that would eliminate the need to manually screen as many as one half of all smears. It was temporarily redesigned as a quality control rescreening device called the AutoPap 300 QC System and obtained FDA approval for this function in 1995. The AutoPap 300 QC System did not find a wide audience, however, and became obsolete in the year 2000. A redesign resulted in a new instrument (the AutoPap System-Primary Screener, later renamed FPSP) which obtained FDA approval as a primary screening device in 1998. In this mode, the device is used in the initial screening of smears. It identifies approximately 25% of slides as requiring “no further review.” Of the remaining slides that require manual review, it also identifies at least 15% for a second manual review, which may be used as a substitute for the 10% review of negative Pap samples required of all U.S. laboratories (see Chapter 17).

A barcode is applied to each slide and slides are loaded into slide trays. Up to 288 slides can be loaded at a time (8 slides per tray, 36 trays). Each slide is analyzed using preset algorithms at $\times 4$ magnification for a visual map of the entire slide, then 1000 fields are captured at $\times 20$ magnification. After analysis, the device assigns a score (from 0 to 1.0) to each slide according to the likelihood of an abnormality. Slides below a cutoff are considered no further review, and those above the cutoff are triaged for full manual review. Any slide deemed unsuitable for analysis because of preparation or cover slipping problems requires manual review.

The accuracy of the instrument was evaluated in a clinical trial at five laboratories.⁵³ Each slide was first evaluated in the conventional manner. The same slides were then processed by the AutoPap System, which detected significantly more abnormal slides—atypical squamous cells of undetermined significance (ASC-US) or greater—than conventional practice (86% versus 79%).

Importantly, FPSP is not approved for women at high risk for cervical cancer. Thus, a laboratory that uses FPSP for primary screening must set aside all Paps from high-risk women for manual screening. It is up to the laboratory to define what constitutes a Pap from a high-risk patient.

False-negative results are occasionally encountered with the FPSP. In the clinical trial, there were 10 false-negatives (5 ASC-US, 4 LSIL, and 1 HSIL) in the 1182 cases considered no further review by FPSP, and Cengel and colleagues found 9 false-negatives (5 ASC-US and 4 LSIL) in the 296 cases considered no further review by FPSP.⁵⁴

The productivity gain with FPSP is modest, because in practice the FPSP archives only about 16% to 17% of Paps without full manual review.^{53,55}

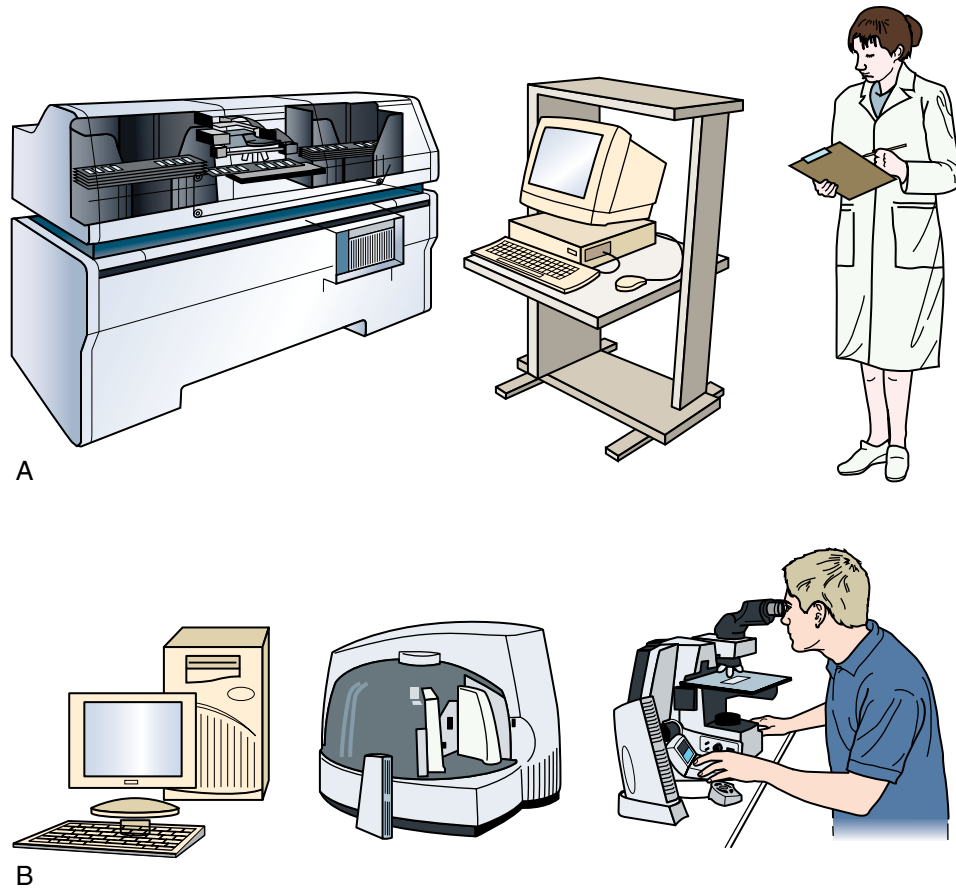


Figure 1.2 Automated cytology screening devices. A, FocalPoint Slide Profiler. The FocalPoint consists of an imaging system and accompanying computer workstation with monitor and keyboard. After imaging is completed, the instrument prints a score for each slide. Depending on the score, the slide is either reported as negative and archived without further review, or it is triaged for manual review. B, ThinPrep Imaging System. The ThinPrep imager consists of two components, a table-top imager and an electronically linked customized review microscope. Slides are imaged on the imager and brought to the microscope for location-guided review.

ThinPrep Imaging System

The ThinPrep Imaging System (TIS) uses location-guided screening to aid the cytotechnologist in reviewing a ThinPrep Pap slide. The TIS consists of two components, the image processor (“imager”) and the review scope (RS; Fig. 1.2B). Stained and cover slipped ThinPrep slides are placed in a cartridge (each cartridge holds 25 slides), and up to 10 cartridges are loaded onto the benchtop imager. The imager has the capacity to screen over 300 slides per day. It scans the slides and identifies 22 fields of view (FOV) on each slide based on optical density measurements and other features. The *x* and *y* coordinates of the 22 FOV are stored in a database and retrieved at a later time. The server is electronically linked to one or more RSs in the laboratory. An RS resembles a standard microscope but is augmented with an automated stage, a pod that controls the stage and objectives, and a keypad. The scope also has a camera that reads the slide identifier when the slide is loaded onto the stage. When a valid slide identifier is recognized, the server sends its coordinate information to the scope, permitting the

cytotechnologist to navigate to the 22 FOV using the pod. Navigation to each FOV is done geographically, that is, using the shortest distance from one FOV to the next. The cytotechnologist uses the pod to advance forward or return back through the FOV, changing objectives as needed. If no abnormal cells are found in any of the FOV, the case has been completed and can be reported as negative. If any abnormal cells are found in any of the FOV, a review of the entire slide must be performed. This can be done using the autoscan function on the RS, with preset, customized user screening preferences. The RS has both electronic and physical slide dotting capabilities.

The accuracy of the TIS was evaluated in a clinical trial at four laboratories. ThinPrep slides were first screened manually and the results recorded. They were then rescreened using the TIS. Truth adjudication was performed by expert review of all abnormal cases and a proportion of negative slides. The TIS detected significantly more abnormal slides (ASC-US or greater) than manual review (82% versus 76%).⁵⁶ A later split-sample study comparing conventional smear cytology versus the TIS

for ThinPrep slides showed a significantly higher detection rate of histologic HSIL (CIN 2,3) with the TIS.⁵⁷

Because 22 FOV represent approximately 25% of the ThinPrep cell spot,⁵⁸ implementation of the TIS comes with a significant productivity enhancement, and in some laboratories the productivity of cytotechnologists has as much as doubled.^{56,59,60}

Implementing the TIS requires adopting the proprietary ThinPrep Pap stain, to which some adjustment is necessary because it yields darker nuclear staining of metaplastic and endocervical cell clusters than most traditional Pap stains. Like FPSP, TIS does not eliminate false-negatives, which are still encountered, albeit less frequently than in the absence of imaging.⁵⁶ A number of postapproval studies have shown significant increases in the detection of LSIL and HSIL after implementation of the TIS.^{61–63}

ACCURACY AND REPRODUCIBILITY

The sensitivity of cytology for detecting preinvasive squamous and glandular lesions is difficult to establish, but it is clearly far from perfect. Most studies of preinvasive lesions suffer from verification bias (i.e., cases are referred for biopsy on the basis of an abnormal smear, and women with negative Pap tests are not biopsied). The few relatively unbiased studies show that the mean sensitivity of the Pap test is 47% (range 30% to 80%), and the mean specificity is 95% (range 86% to 100%).⁶⁴

The sensitivity of cytology is less than ideal for invasive cancers as well, and estimates range widely (16% to 82%). Many women with cervical cancer have a history of one or more negative smears.^{65–76} The relative contributions of sampling and laboratory error vary from one study to another and likely depend on how carefully retrospective rescreening is performed.

False-positive diagnoses of cervical cancer occur in 10% to 15% of cases.^{77,78} The chief culprits are the atrophic smear with benign squamous atypia in a granular, pseudonecrotic background; reparative changes; and keratinizing HSILs.

The interobserver reproducibility of cytologic interpretations is less than perfect. In a large study of women, most of whom had mild cytologic abnormalities, the unweighted κ statistic for four categories of diagnosis—negative, atypical, LSIL, and HSIL—was 0.46, indicating moderate reproducibility.⁷⁹ (Roughly, a κ of 0 or less represents poor agreement, 0 to 0.2 slight agreement, 0.2 to 0.4 fair agreement, 0.4 to 0.6 moderate agreement, 0.6 to 0.8 very good agreement, and 0.8 to 1.0 almost perfect agreement.) In the same study, the reproducibility of histologic interpretations of cervical biopsies, also for four categories of diagnosis, was identical (0.46). The greatest disagreement with Pap tests involved those originally

interpreted as showing ASC-US; the second reviewer agreed with only 43% of cases. The greatest disagreement with biopsies involved those originally interpreted as LSIL; the second reviewer concurred in only 43% of cases.⁷⁹

A graphic demonstration of the relative reproducibility of various cytologic findings is available on the Bethesda System Web Atlas, which contains the results of the Bethesda Interobserver Reproducibility Project. A large number of images were reviewed by hundreds of observers, who were asked to place the images into one of the Bethesda System categories. The results are displayed for each image as a histogram.⁸⁰

DIAGNOSTIC TERMINOLOGY AND REPORTING SYSTEMS

Papanicolaou devised a numerical system for reporting cervical smears, which was originally intended to convey his degree of suspicion that the patient had cancer: **class I**, absence of atypical or abnormal cells; **class II**, atypical but no evidence of malignancy; **class III**, suggestive of but not conclusive for malignancy; **class IV**, strongly suggestive of malignancy; and **class V**, conclusive for malignancy. Over time, however, the Papanicolaou class system underwent many modifications and was not used in a uniform fashion.⁸¹ It persisted in many laboratories well into the 1980s, however. In other laboratories it was replaced (or supplemented) by descriptive terms borrowed from histologic classifications of squamous lesions. Squamous cancer precursors were originally divided into **carcinoma in situ**, which was a high-risk lesion of immature, undifferentiated atypical cells, and **dysplasia** (subdivided into mild, moderate, and severe), considered to be a low-risk lesion composed of more mature squamous cells. In the 1960s, Richart challenged the duality of dysplasia/carcinoma in situ and proposed a new term, **cervical intraepithelial neoplasia** (CIN). CIN was graded from 1 to 3, but Richart believed that CIN 1 (mild dysplasia) had a strong propensity to progress to CIN 3 and cancer. The high rate of progression found in his study most likely related to stringent entry criteria; for inclusion, CIN 1 had to be confirmed on three consecutive Paps.⁸² Richart's data showed a higher progression rate for mild dysplasia than most other natural history studies.⁸³ The CIN concept was highly influential, however, and for many years squamous precursors were treated as much on the basis of their size and location as on their grade. This situation remained for two decades.

In 1989 the Bethesda System was introduced to standardize the reporting of cervical cytology results and incorporate new insights gained from the discovery of HPV.⁸⁴ The name for a squamous cancer precursor was changed to **squamous intraepithelial lesion** (SIL), subdivided into only two grades (low and high) based on the

evolving understanding of the biology of HPV. In this system, LSIL encompasses CIN 1, and HSIL encompasses CIN 2 and 3. This was a shift away from the CIN concept, one based on a reevaluation of the existing evidence, which demonstrated that most LSILs are, in fact, transient HPV infections that carry little risk for oncogenesis, whereas most HSILs are associated with viral persistence and a significant potential for progression to invasive cancer.

The first Bethesda System workshop in 1988 was followed by two others in 1991 and 2001, which made modifications to the original framework and terminology. The 2001 workshop broadened participation by using a dedicated Web site on the Internet, and an electronic bulletin board received more than 1000 comments regarding draft recommendations. The 2001 Bethesda System, like its predecessors, recommends a specific format for the cytology report, starting with an explicit statement on the adequacy of the specimen, followed by a general categorization and an interpretation or result.^{85,86}

THE BETHESDA SYSTEM

Specimen Adequacy

One of the most important advances of the Bethesda System is its recommendation that each Pap report begin with a statement of adequacy. In 1988, the Bethesda System proposed three categories for specimen adequacy: “satisfactory,” “less than optimal” (renamed “satisfactory but limited by ...” in 1991), and “unsatisfactory.” The 2001 Bethesda System eliminated the middle category because it was confusing to physicians and prompted unnecessary repeat Pap tests. Nevertheless, the 2001 Bethesda System advocates mentioning the presence or absence of a transformation zone component and permits comments on obscuring elements. The 2001 Bethesda System criteria for adequacy are listed in Table 1.1. They are somewhat arbitrary, because scientific data on adequacy are limited, particularly regarding the minimum number of cells needed for an adequate sample.

It is easy to determine whether a specimen is adequate or unsatisfactory in most cases. Slides received without patient identification or broken beyond repair should be rejected as unsatisfactory. An appropriately labeled smear with an adequate complement of well-preserved squamous and endocervical cells is clearly satisfactory. On average, about 0.5% of Pap samples are interpreted as unsatisfactory.⁸⁷ Unsatisfactory Pap samples can be finalized by a cytotechnologist and need not be reviewed by a cytopathologist (see Chapter 17).

One of the components of an adequate smear is an adequate squamous component. In the 1988 and 1991 Bethesda Systems, the requirement for an adequate squamous component was defined as “well-preserved

TABLE 1.1 THE 2001 BETHESDA SYSTEM CATEGORIES FOR SPECIMEN ADEQUACY

Satisfactory for Evaluation

A satisfactory squamous component must be present (see text).

Note the presence or absence of endocervical or transformation zone component.

Obscuring elements (inflammation, blood, drying artifact, other) may be mentioned if 50% to 75% of epithelial cells are obscured.

Unsatisfactory for Evaluation

Specimen rejected or not processed because (specify reason). Reasons may include:

- lack of patient identification.
- unacceptable specimen (e.g., slide broken beyond repair).

or:

Specimen processed and examined, but unsatisfactory for evaluation of an epithelial abnormality because (specify reason). Reasons may include:

- insufficient squamous component (see text).
- obscuring elements cover more than 75% of epithelial cells

and well-visualized squamous epithelial cells should cover more than 10% of the slide surface.”⁸⁸ This guideline, however, was interpreted differently by different cytologists. Even in laboratories that interpreted it literally, observers consistently overestimated the percentage of slide coverage by squamous cells.⁸⁹ With the 2001 Bethesda System modification, the requirement was redefined as a minimum “estimated number of squamous cells,” the minimum being different for conventional versus liquid-based preparations.

The minimum number of squamous cells for adequacy depends on the preparation method:

- liquid-based: 5000
- conventional: 8000 to 12,000

The minimum number of 5000 squamous cells for an adequate LBC Pap was based on correlations made between the false-negative rate and squamous cell cellularity.⁹⁰ Because LBCs likely represent a more homogeneous representation of the material obtained by the collection device,⁹¹ a more stringent squamous cellularity requirement was imposed on conventional smears.

Whether or not a slide contains an adequate squamous cell component is immediately apparent in most cases. In borderline cases, techniques are available for estimating adequacy: reference images for conventional smears and a spot-counting procedure for liquid-based preparations. Reference images of known cell counts are useful for estimating cellularity.⁸⁹ Because of this, the 2001 Bethesda System published images to assist in the estimation of squamous cellularity on conventional smears.⁸⁶

A spot-counting method is used to evaluate LBCs with borderline squamous cellularity. A minimum of 10 fields are counted along a diameter that includes the center of the slide (Fig. 1.3A). If the cell circle has blank spots, these should be represented in the fields counted (Fig. 1.3B). The average number of squamous cells is then compared against tables that take into account the objective, the eyepiece field number, and the diameter of the circle that contains cellular material.⁸⁶ For example, with an FN20 eyepiece, and a $\times 40$ objective, the sample is adequate if the average number of cells counted is greater than 3.1 for a ThinPrep slide.

Additional slides can usually be generated from the residual vial of an LBC sample. In some laboratories, an additional slide is prepared when the initial slide has insufficient cellularity. The addition of a washing step with 10% glacial acetic acid increases the percentage of satisfactory ThinPrep Pap samples, uncovering occasional cases of SIL and invasive cancer.^{92,93}

The cellularity of the squamous cell component is *estimated*; laboratories are not expected to count individual cells. Squamous cellularity is sometimes particularly difficult to estimate, for example, when there is marked cell clustering or cytolysis. In certain clinical settings, particularly in women with atrophy, a lower number may be adequate. In these situations, cytologists are expected to use their judgment when evaluating adequacy.⁸⁶

In the 2001 Bethesda System, the presence or absence of an endocervical or transformation zone component is noted on the report. An endocervical component is considered present if 10 or more endocervical or squamous

metaplastic cells, either isolated or in groups, are present. The data on the endocervical component as a measure of adequacy are contradictory.⁹⁴ The importance of endocervical cells was first suggested by cross-sectional studies, which showed that smears are more likely to contain SIL when endocervical cells are present.⁹⁵⁻⁹⁷ Data from retrospective case-control studies, however, do not support this; investigators have found no association between false-negative Pap samples and the absence of endocervical cells.^{98,99} Retrospective cohort studies have shown that women whose initial smears lack endocervical cells do not develop more lesions on follow-up than women whose smears do have an endocervical component,¹⁰⁰⁻¹⁰² implying that an endocervical component is not essential. Currently, a smear without endocervical cells is not considered unsatisfactory, although the absence of an endocervical or transformation zone component is mentioned as a “quality indicator.” This is not to imply that a repeat Pap is necessary. Physicians are expected to use their judgment and to consider repeating the Pap if the patient is at high risk for cervical cancer.

General Categorization

The general categorization is an optional component of the 2001 Bethesda System.

Three categories:

- negative for intraepithelial lesion or malignancy
- epithelial cell abnormality
- other

The 1991 Bethesda categories “within normal limits” and “benign cellular changes” were combined into a single “negative” category in 2001. “Other” includes cases that do not fit neatly into one of the other two categories: non-epithelial malignancies, such as melanoma and lymphoma, and benign-appearing endometrial cells in women over 40 years of age.

Specimens are categorized according to the most significant abnormality identified.

Interpretation and Results

Recommended terminology for reporting findings is listed in Table 1.2.

Non-neoplastic findings, other than organisms, are optional, given that many physicians desire the Pap test report to be as concise as possible. Findings of no clinical consequence, if mentioned, may result in confusion and even unnecessary repeat testing. Nevertheless, many cytologists believe it is important to document that certain findings were interpreted as benign, particularly those that can mimic a neoplasm.

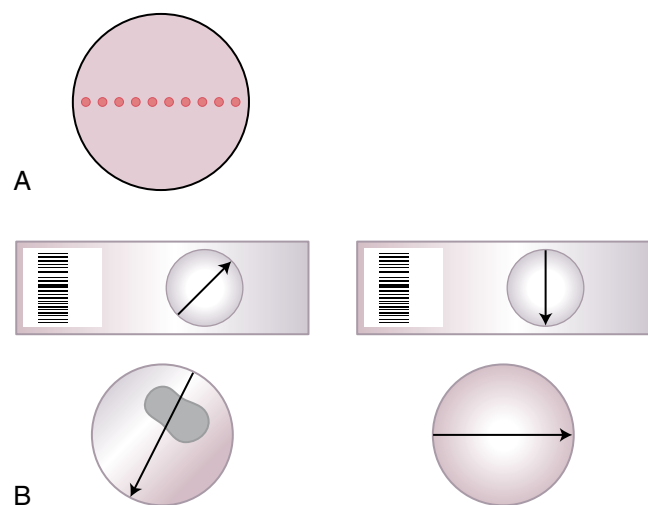


Figure 1.3 Method for estimating the adequacy of the squamous component of liquid-based preparations. A, At $\times 40$, 10 fields are counted starting at the edge (horizontal or vertical) and including the center of the preparation. B, An attempt is made to include “holes” in proportion to their size, making sure that the fields counted cover both cellular and sparsely cellular areas in proportion to their size.

TABLE 1.2 THE 2001 BETHESDA SYSTEM**Specimen Adequacy (see Table 1.1)****General Categorization (Optional)**

Negative for intraepithelial lesion or malignancy (NILM)

Epithelial cell abnormality

Other

Interpretation/results

NILM

Organisms

Trichomonas vaginalis

Fungal organisms morphologically consistent with

Candida species

Shift in flora suggestive of bacterial vaginosis

Bacteria morphologically consistent with *Actinomyces* species

Cellular changes consistent with herpes simplex virus

Other non-neoplastic findings (optional to report; list not comprehensive)

Reactive cellular changes associated with: inflammation (includes typical repair); radiation; intrauterine contraceptive device (IUD)

Glandular cells status post hysterectomy

Atrophy

Epithelial cell abnormalities

Squamous cell

Atypical squamous cells (ASC)

- of undetermined significance (ASC-US)

- cannot exclude HSIL (ASC-H)

Low-grade squamous intraepithelial lesion (LSIL)

High-grade squamous intraepithelial lesion (HSIL)

Squamous cell carcinoma (SCC)

Glandular cell

Atypical glandular cells (AGC); specify endocervical, endometrial, or not otherwise specified

AGC, favor neoplastic (specify endocervical or not otherwise specified)

Endocervical adenocarcinoma in situ (AIS)

Adenocarcinoma

Other

Endometrial cells in a woman older than 40 years of age

Automated Review and Ancillary Testing (Include as Appropriate)**Educational Notes and Suggestions (Optional)**

In the United States, a pathologist is required to review cases that show reactive or reparative changes and any abnormality at the level of ASC-US or higher. This represents about 10% to 20% of the total Pap volume in most laboratories.

Squamous Cells

The ectocervix is lined by a stratified squamous epithelium that matures under the influence of estrogen. The most mature squamous cells are called *superficial cells*. They have a small, pyknotic nucleus that is 5 to 6 μm in diameter. *Intermediate cells* have a larger nucleus measuring 8 μm in diameter, which is not pyknotic but instead has a finely granular texture. Intermediate cells are occasionally binucleated and even multinucleated. Both superficial and intermediate cells are large polygonal cells with transparent pink or green cytoplasm (Fig. 1.4). Superficial and intermediate cells are the predominant cells in cytologic samples from women of reproductive age.

Immature squamous cells are called *parabasal cells* and *basal cells*. Because a Pap test does not usually scrape off the entire thickness of the epithelium but only the upper few layers, immature cells near the base of a mature epithelium are not sampled. An immature epithelium, however, is composed throughout its thickness by parabasal-type cells or basal-type cells. Immature epithelium is common at the transformation zone, where it is called squamous metaplasia, and whenever there is squamous epithelial atrophy as a result of a low estrogen state. Thus, parabasal and basal cells are typically obtained from squamous metaplasia or atrophic epithelium.

Squamous atrophy is encountered in a variety of clinical settings associated with a low estrogen state.

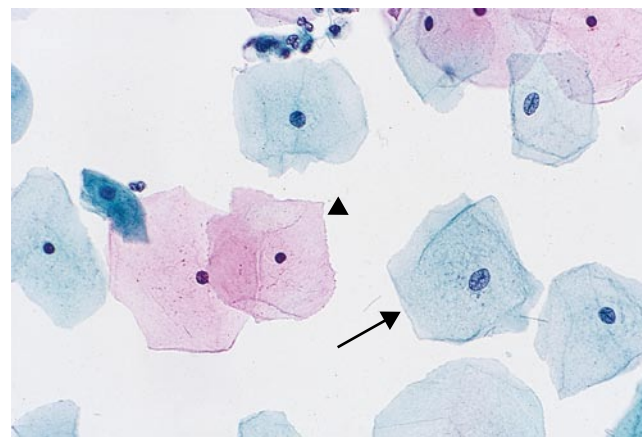


Figure 1.4 Superficial and intermediate squamous cells. The mature squamous epithelium of the ectocervix in women of reproductive age is composed throughout most of its thickness by superficial (arrowhead) and intermediate (arrow) cells.

THE NORMAL PAP

A normal Pap test result begins with a statement of adequacy, followed by “negative for intraepithelial lesion or malignancy” (NILM). Additional findings (e.g., reactive changes, infectious organisms) are listed subsequently. Approximately 91% of Pap tests are interpreted as such.⁸⁷ Normal Pap tests, with the exception of those cases that show reactive or reparative changes, can be finalized by a cytotechnologist and need not be reviewed by a pathologist (see Chapter 17).

Low estrogen states include:

- premenarche
- postpartum
- postmenopause
- Turner syndrome
- status post bilateral oophorectomy

Immature, parabasal cells are round or oval rather than polygonal and have a variably sized nucleus that is usually larger than that of an intermediate cell. Basal cells are even smaller and have scant cytoplasm (Fig. 1.5).

Basal and parabasal cells are the hallmark of atrophy. With a deeply atrophic cervical epithelium, no superfi-

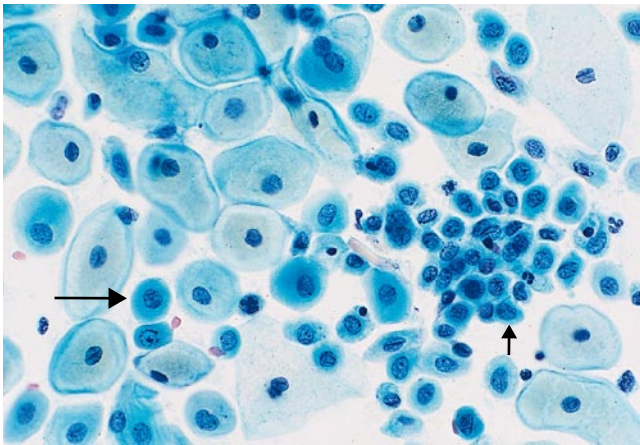


Figure 1.5 Parabasal and basal cells (postpartum smear). Parabasal cells (*large arrow*) are oval and typically have dense cytoplasm. Basal cells (*small arrow*) are similar but have less cytoplasm. Many cells have abundant pale-yellow staining glycogen, a characteristic but nonspecific feature of squamous cells of pregnancy and the postpartum period.

cial or intermediate cells are seen, only parabasal and basal cells. In addition, atrophic epithelium, particularly in postmenopausal women, is prone to injury and inflammation and often shows a spectrum of changes that must be recognized as normal and not confused with a significant lesion. The sheets of immature cells are crowded and syncytium-like, mimicking the crowded cells of an HSIL (Fig. 1.6). Nevertheless, the chromatin texture in atrophy is finely granular and evenly distributed, and nuclear contours remain mostly smooth and thin. A curious variant, *transitional cell metaplasia*, is notable for prominent longitudinal nuclear grooves (“coffee-bean nuclei”), wrinkled nuclei, and small perinuclear halos (Fig. 1.6B).¹⁰³ Cellular degeneration is seen in some cases of atrophy (Fig. 1.7A). Air-drying, a common artifact with smears, causes artificial nuclear enlargement. Dark blue, rounded, amorphous masses known as “blue blobs,” thought to represent either condensed mucus or degenerated bare nuclei, are sometimes seen (Fig. 1.7B), as is a granular background (see Fig. 1.7A) that resembles the necrosis associated with invasive cancers.

Parabasal cells are also the constituents of squamous metaplasia of the endocervix. Squamous metaplasia is a common morphologic alteration of the endocervical epithelium usually limited to the transformation zone in women who otherwise have good squamous maturation. It is identified on smears as flat sheets of immature squamous cells (parabasal cells), often arranged in an interlocking fashion like paving stones (Fig. 1.8). The parabasal cells may show mild variation in nuclear size, with slightly irregular contours and slight hyperchromasia.

Squamous metaplasia, as defined cytologically, is always composed of parabasal cells (immature squamous cells). So-called mature squamous metaplasia, a

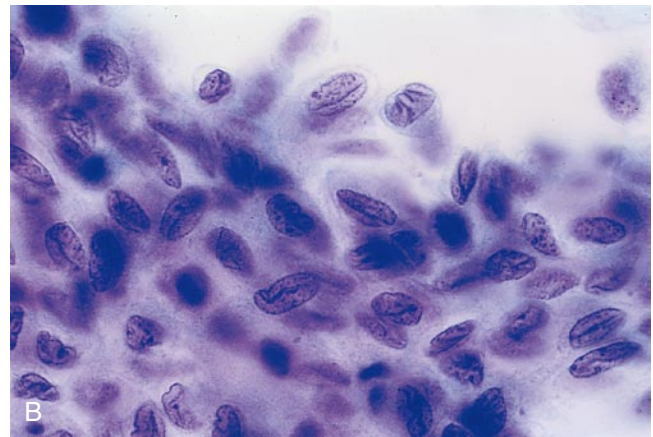
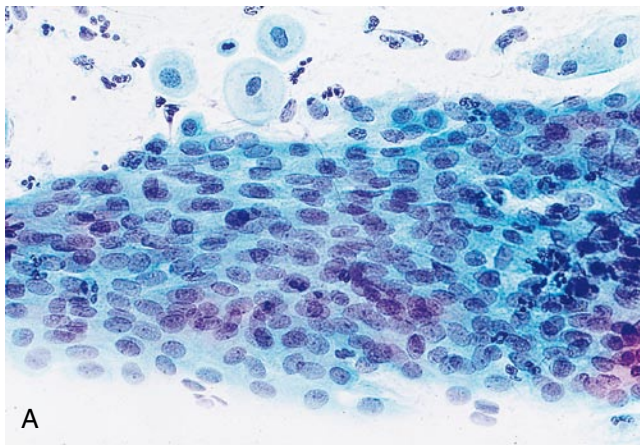


Figure 1.6 Parabasal cells (postmenopausal smear). A, Atrophic epithelium is composed almost exclusively of parabasal cells, often arranged in broad, flowing sheets. B, Transitional cell metaplasia. In this uncommon condition, the atrophic epithelium resembles transitional cell epithelium by virtue of its longitudinal nuclear grooves. Nuclear membrane irregularities raise the possibility of a high-grade squamous intraepithelial lesion (HSIL), but the chromatin is pale and finely textured.

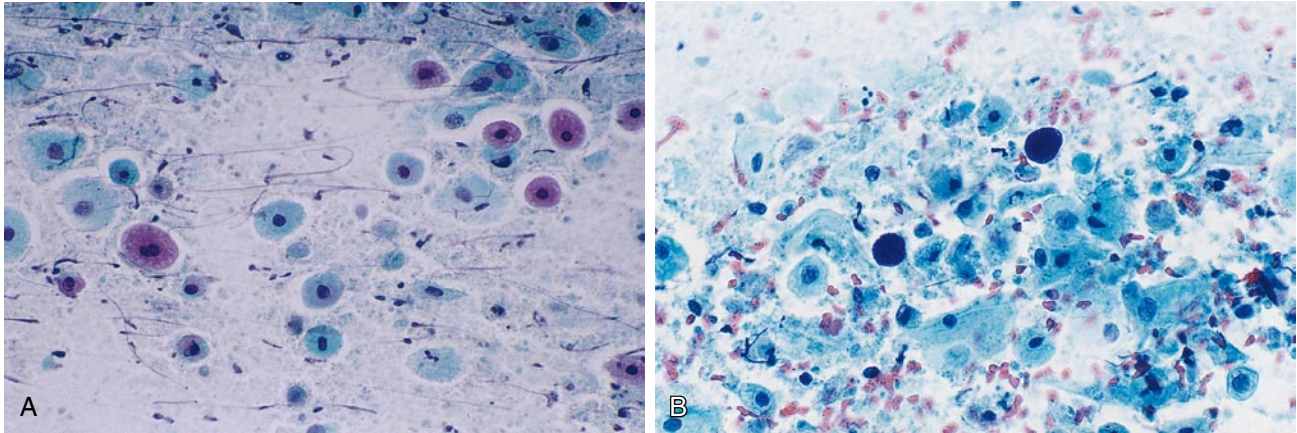


Figure 1.7 Parabasal cells (postmenopausal smear). A, Degenerated parabasal cells in atrophic smears have hyper eosinophilic cytoplasm and a pyknotic nucleus. Note the granular background, which is commonly seen in normal atrophic smears. B, Dark blue blobs are seen in some atrophic smears. These featureless structures should not be interpreted as a significant abnormality.

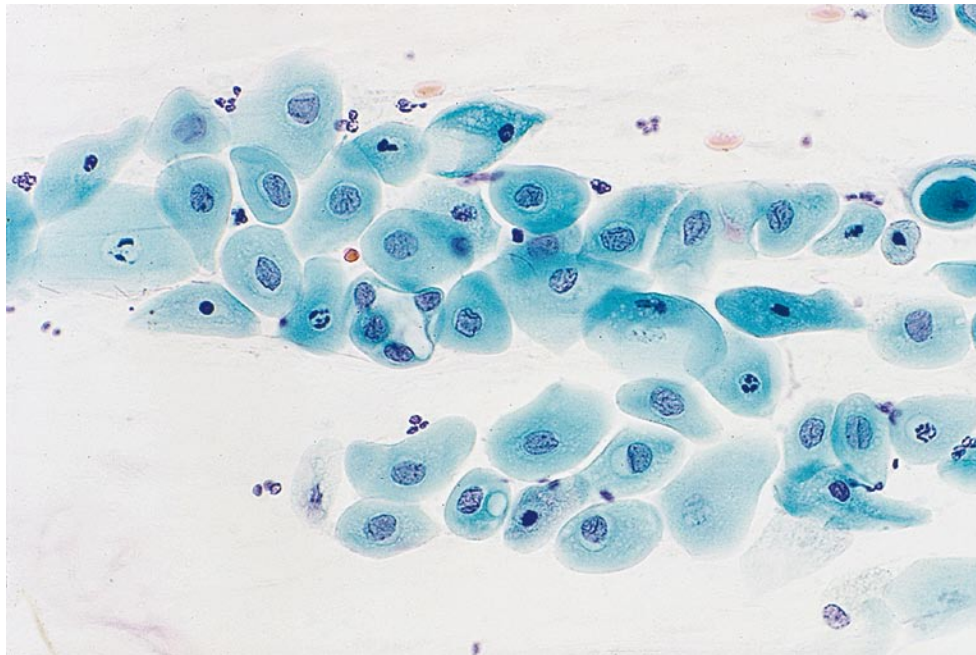


Figure 1.8 Squamous metaplasia. Interlocking parabasal-type cells, as seen here, represent squamous metaplasia of the endocervix.

histologic term describing mature squamous epithelium overlying endocervical glands, is not recognized as such on cytologic preparations.

Other normal changes of squamous cells are hyperkeratosis and parakeratosis. *Hyperkeratosis* is a benign response of stratified squamous epithelium as a result of chronic mucosal irritation, as in uterine prolapse. Anucleate, mature, polygonal squamous cells appear as isolated cells or plaques of tightly adherent cells (Fig. 1.9A). Such cells are benign and should not be considered abnormal. This cytologic picture is mimicked by contamination of the slide by squamous cells of the vulva or skin from the fingers of the persons handling the slide.

Parakeratosis, a benign reactive change also caused by chronic irritation, is characterized by small, heavily keratinized squamous cells with dense orangeophilic cytoplasm and small, pyknotic nuclei (Fig. 1.9B). When such densely keratinized cells show nuclear atypia in the form of enlargement and membrane irregularity with hyperchromasia, they are called “dyskeratocytes” or “atypical parakeratosis” and should be categorized as an epithelial cell abnormality.

Endocervical Cells

The endocervix is lined by a mucin-producing columnar cell that has an eccentrically placed nucleus with a finely

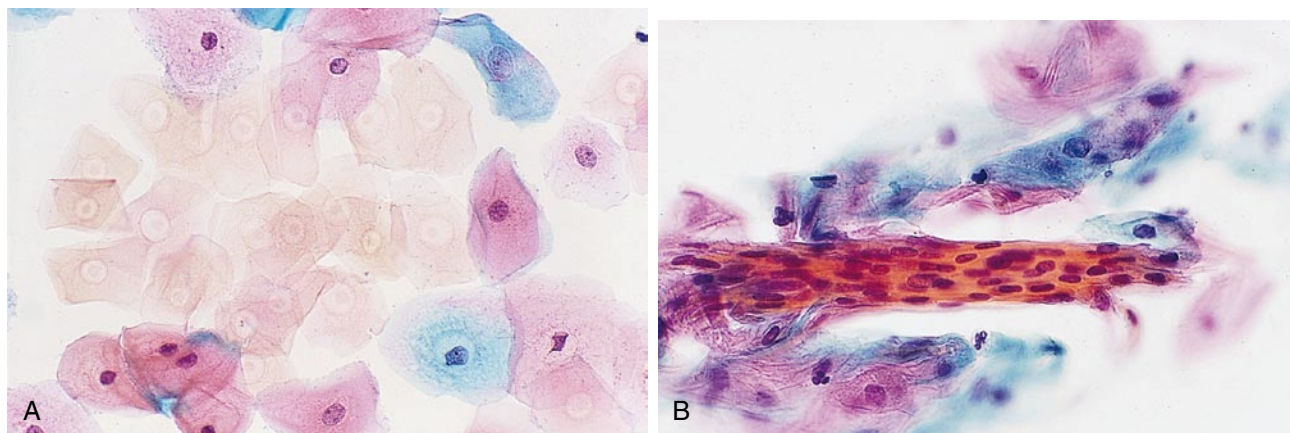


Figure 1.9 Keratosis. A, Hyperkeratosis. Anucleate squames are a protective response of the squamous epithelium. B, Parakeratosis. Parakeratosis appears as plaques, as seen here, or as isolated cells.

granular chromatin texture and abundant vacuolated cytoplasm. Nucleoli are inconspicuous but become quite prominent in reactive conditions, such as cervicitis (see section on reactive changes). Endocervical cells are often identified in strips or sheets rather than as isolated cells (Fig. 1.10). When arranged as strips, the cells have the appearance of a picket fence. When in sheets, they resemble a honeycomb because of the well-defined cell borders and uniform cell arrangement. Rarely, mitoses are identified. They should not raise suspicion of a neoplasm if the cells are otherwise normal in appearance. Tubal metaplasia is a benign alteration of the endocervical epithelium found in about 30% of cone biopsy and hysterectomy specimens (Fig. 1.11).¹⁰⁴

Exfoliated Endometrial Cells

Spontaneously exfoliated, menstrual endometrial cells are seen if the Pap is taken during the first 12 days of the menstrual cycle.¹⁰⁵

CYTOMORPHOLOGY OF EXFOLIATED ENDOMETRIAL CELLS:

- balls of small cells
- isolated small cells
- scant cytoplasm
- dark nucleus
- nuclear molding
- nuclear fragmentation

Exfoliated endometrial cells are most easily recognized when they are arranged in spherical clusters (Fig. 1.12). They are small cells with a dark nucleus and (usually) scant cytoplasm. Occasional cells may have more abundant clear cytoplasm. Clusters have a scalloped contour as a result of the slight protrusion of individual cells. Apoptosis is common. Isolated endometrial cells are also seen, but they are less conspicuous because of their small size.

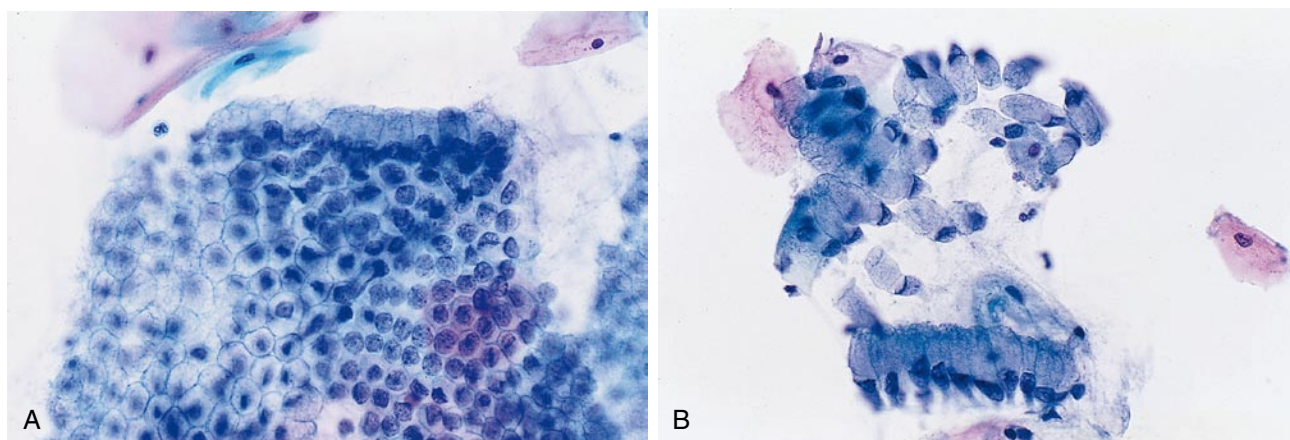


Figure 1.10 Endocervical cells. A, Normal endocervical cells are often arranged in cohesive sheets. Note the even spacing of the nuclei, their pale, finely granular chromatin, and the honeycomb appearance imparted by the sharp cell membranes. B, Sometimes they appear as strips or isolated cells. Abundant intracytoplasmic mucin results in a cup-shaped nucleus.

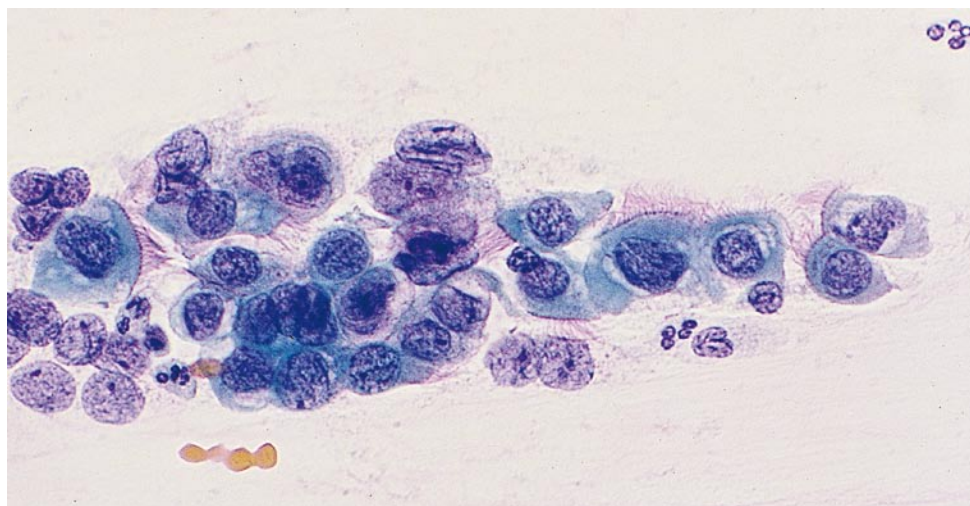


Figure 1.11 Tubal metaplasia. Ciliated endocervical cells are occasionally seen.

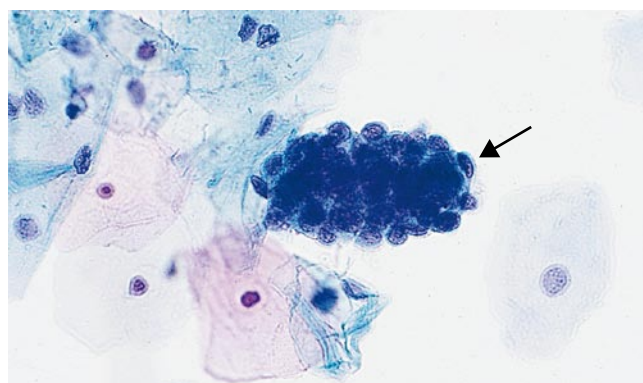


Figure 1.12 Endometrial cells. Spontaneously exfoliated endometrial cells, as in menses, are small cells arranged in balls. Cytoplasm is scant. Nuclei around the perimeter appear to be wrapping around adjacent cells (arrow), a characteristic but non-specific feature.

Occasionally, endometrial cell clusters consist of an obvious dual cell population with small, dark stromal cells (in the center) and larger glandular cells (around the edges). Most endometrial cell clusters, however, do not have this dual population. “Monocontoured clusters” like that in [Figure 1.12](#) may consist of glandular endometrial cells, stromal endometrial cells, or a mix of both.¹⁰⁶

Shedding endometrial cells after day 12 (“out of phase”) is associated with endometritis, endometrial polyps, and intrauterine devices (IUDs). In a young woman, abnormal shedding is almost never a result of endometrial adenocarcinoma.^{107,108} For this reason, endometrial cells do not need to be mentioned in the report for women under 40 years of age. Some laboratories do so anyway, to document that the cells were identified and interpreted as benign endometrial cells. Endometrial cells are notorious for their ability to cause diagnostic difficulty, because a variety of neoplastic cells resemble endometrial cells.

In a woman 40 years of age or older, benign-appearing endometrial cells are reported because of the small associated risk of endometrial neoplasia.

DIFFERENTIAL DIAGNOSIS OF EXFOLIATED ENDOMETRIAL CELLS:

- HSIL
- squamous cell carcinoma
- AIS
- small cell carcinoma

The differential diagnosis includes a number of significant lesions that mimic endometrial cells and thus are sometimes mistakenly interpreted as normal, particularly if the woman is in the first 12 days of her menstrual cycle. Attention to certain cytologic details can help avoid some if not all of these misattributions. A minority of HSILs are composed of relatively small cells. Like endometrial cells, their nuclei are dark, and they have scant cytoplasm ([Fig. 1.13A](#)). HSIL cells, even when small, are usually bigger than endometrial cells, vary more in size, and have denser cytoplasm. HSIL clusters are usually less well circumscribed and are not as spherical as endometrial cell balls. Some poorly differentiated squamous cell carcinomas (SQCs) are composed of small dark cells that mimic endometrial cells to perfection ([Fig. 1.13B](#)). In such cases, suspicious clinical findings (e.g., postcoital bleeding) might be the only clue to the correct interpretation. Most AIS have a columnar cell morphology, but a minority are made up of smaller and rounder cells ([Fig. 1.13C](#)), particularly on LBC preparations. Careful examination for focal columnar differentiation and mitoses can be quite helpful. The rare small cell carcinoma of the cervix may display crush artifact ([Fig. 1.13D](#)), which is rarely seen with endometrial cells.

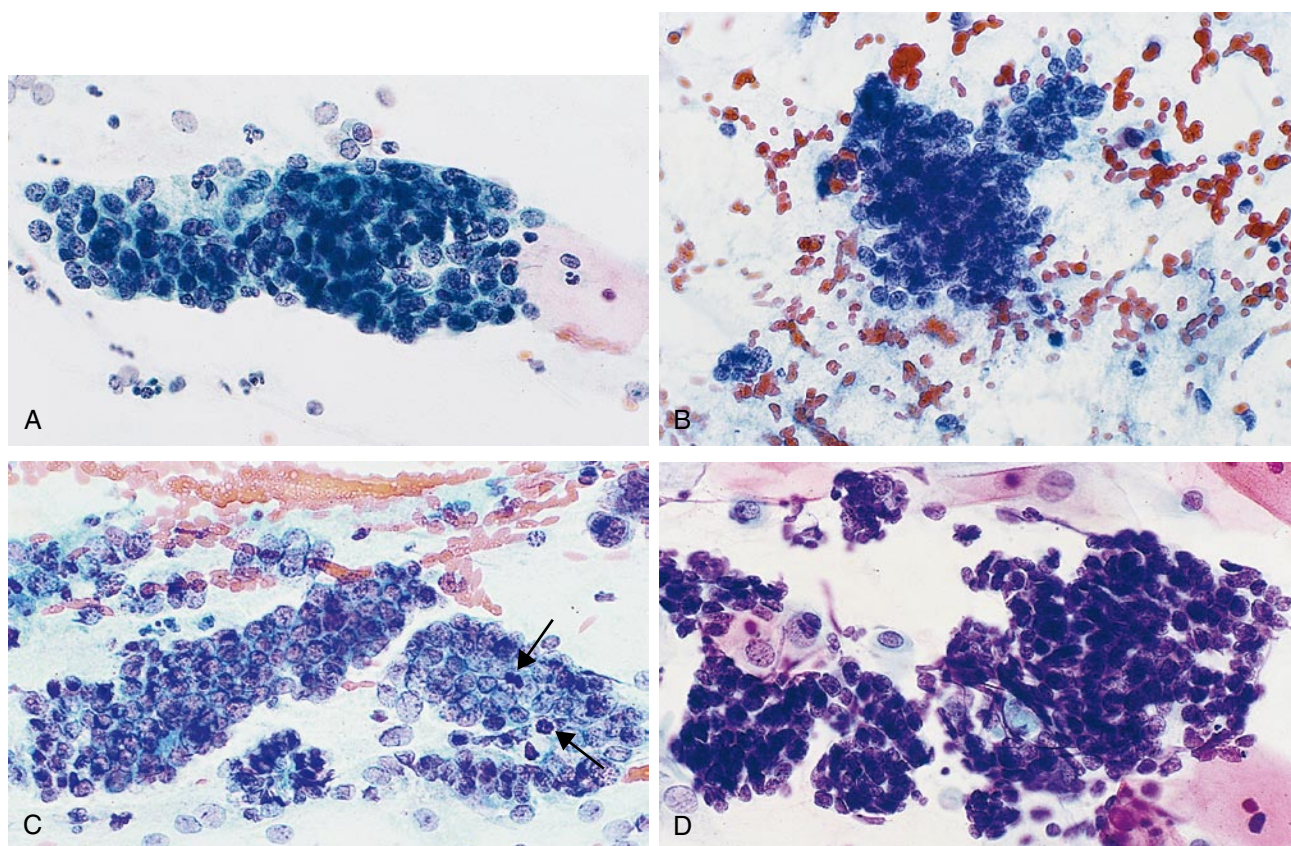


Figure 1.13 Mimics of exfoliated endometrial cells. *A*, High-grade squamous intraepithelial lesion (HSIL). The cells of some HSILs are small but still larger than endometrial cells and usually arranged in flatter aggregates rather than spheres. *B*, Squamous cell carcinoma (SCC). Some poorly differentiated SCCs are indistinguishable from endometrial cells. The granular debris (tumor diathesis) seen here can also be seen in normal menstrual Pap samples. *C*, Adenocarcinoma in situ (AIS). Some cases of AIS have an endometrioid appearance, but mitoses (arrows) are distinctly uncommon in exfoliated endometrial cells. *D*, Small cell carcinoma. The cells resemble endometrial cells but are even darker. There is nuclear smearing, which is rarely seen with benign endometrial cells.

Abraded Endometrial Cells and Lower Uterine Segment

The endocervical sampling device occasionally inadvertently samples the LUS or endometrium.¹⁰⁹ This is especially likely when the endocervical canal is abnormally shortened, as it is after a cone biopsy.¹¹⁰

CYTOMORPHOLOGY OF ABRADED ENDOMETRIUM AND LOWER UTERINE SEGMENT:

- large and small tissue fragments
- glands and stroma
- stromal cells
 - uniform
 - oval or spindle shaped
 - finely granular chromatin
 - occasional mitoses
 - capillaries traverse larger fragments

- glands
 - tubular
 - straight or branching
 - mitoses (some cases)
 - extreme nuclear crowding
 - scant cytoplasm

The characteristic feature is the combination of glands and stroma, often in large fragments (Fig. 1.14A-C), either together or separated. Glandular cells of the LUS resemble endocervical cells, but have a higher nuclear to cytoplasmic ratio, are more hyperchromatic, and can be mitotically active. Because of their high nuclear to cytoplasmic ratio, they can be confused with a significant squamous or glandular lesion.¹⁰⁹

Trophoblastic Cells and Decidual Cells

Syncytiotrophoblastic cells from placental tissue are seen rarely, perhaps in about 0.1% of smears from pregnant

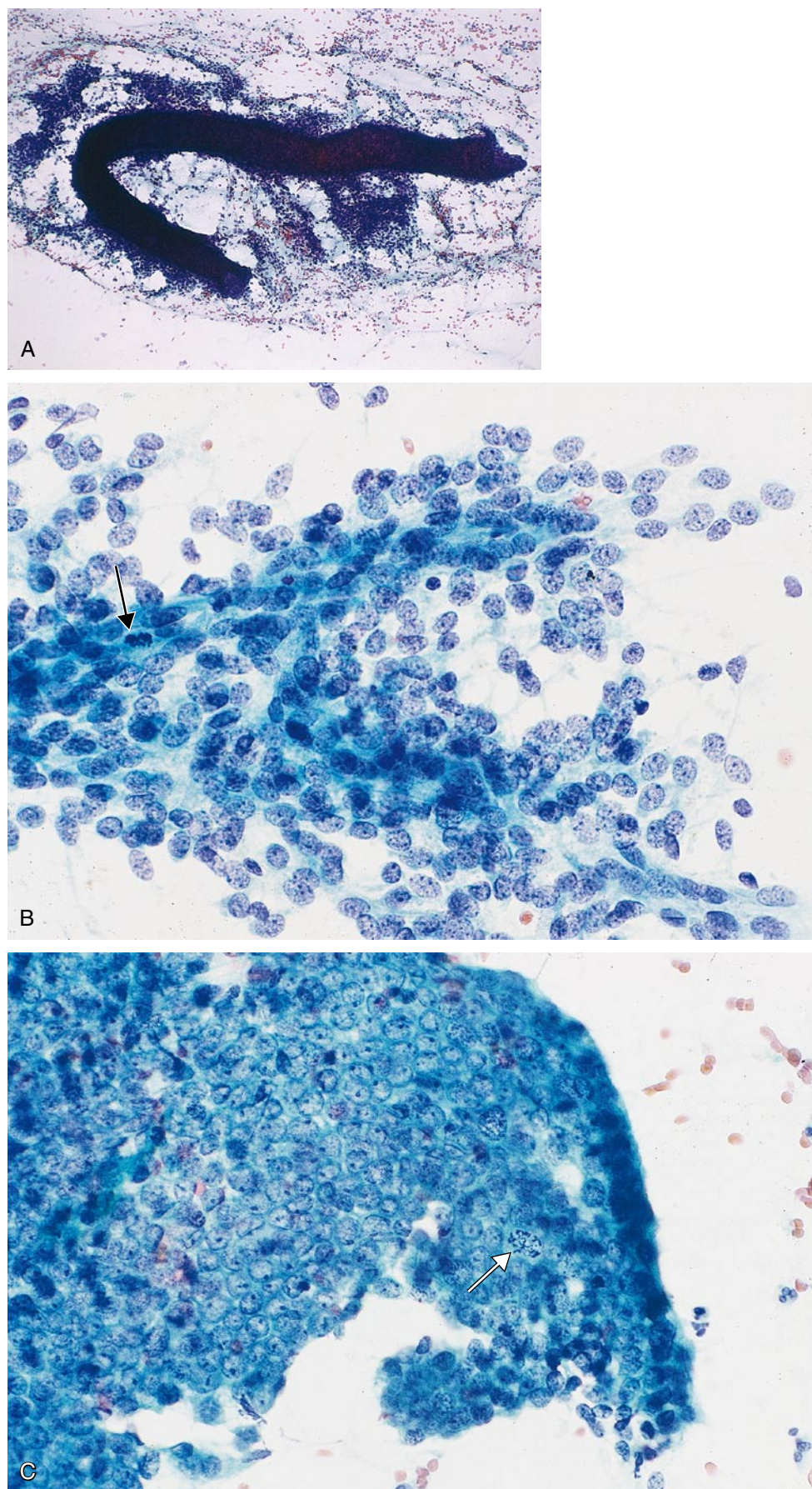


Figure 1.14 Endometrial cells, directly sampled. *A*, An intact endometrial tubule is surrounded by well-preserved endometrial stromal cells. *B*, Benign stromal cells are elongated and mitotically active (arrow) and may suggest a high-grade squamous intraepithelial lesion (HSIL) or a malignancy. The pale, finely granular chromatin and the association with intact endometrial glands are clues to a benign diagnosis. *C*, The glandular cells are crowded and mitotically active (arrow), but evenly spaced.

women.¹¹¹ The cells are large, with abundant blue or pink cytoplasm. They have multiple nuclei that have a granular chromatin texture and slightly irregular contours. Trophoblastic cells can be distinguished from multinucleated histiocytes because their nuclei are darker and more irregular in contour (Fig. 1.15). They do not show the prominent molding and ground-glass appearance of nuclei of herpes simplex infection. Immunostains for human chorionic gonadotropin and human placental lactogen can be used to confirm their identity as trophoblastic cells. The presence of syncytiotrophoblastic cells is not a reliable predictor of an impending abortion.¹¹¹

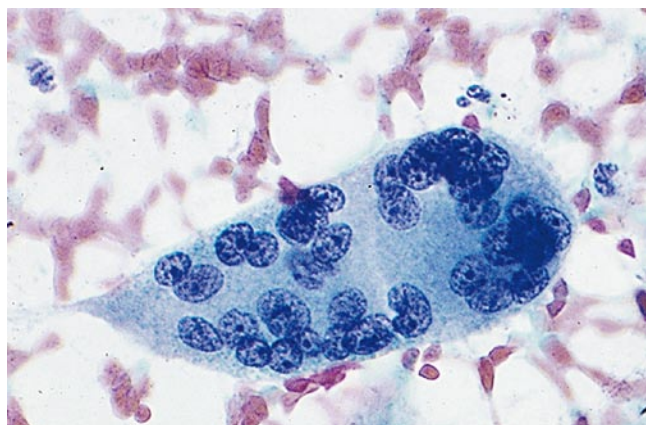


Figure 1.15 Syncytiotrophoblast. The nuclei of these multinucleated cells are dark and coarsely granular, unlike those of histiocytes.

Decidual cells are isolated cells with abundant granular cytoplasm, a large vesicular nucleus, and a prominent nucleolus. They often show degenerative changes.

Inflammatory Cells

Neutrophils are seen in all Pap samples and do not necessarily indicate infection, but they are present in increased numbers after injury or infection. Lymphocytes and plasma cells are rare, but occasionally—most often in older women—they are numerous (Figs. 1.16, 1.71A). This pattern is called *follicular cervicitis* because biopsies show lymphoid follicle formation. The lymphocytes of follicular cervicitis can be confused with HSIL cells, endometrial cells, and lymphoma. Histiocytes are associated with a myriad of conditions (e.g., menses, pregnancy, foreign bodies, radiotherapy, and endometrial hyperplasia and carcinoma) (Fig. 1.17), but by themselves are a nonspecific finding of no clinical significance.

Lactobacilli

The vagina is colonized by gram-positive rod-shaped bacteria of the genus *Lactobacillus*. They are beneficial because they produce lactic acid, which reduces the ambient acid-base balance (pH) and possibly protects from infection by *Candida* and other pathogens. Lactobacilli metabolize the glycogen contained within exfoliated squamous cells. The resulting cellular

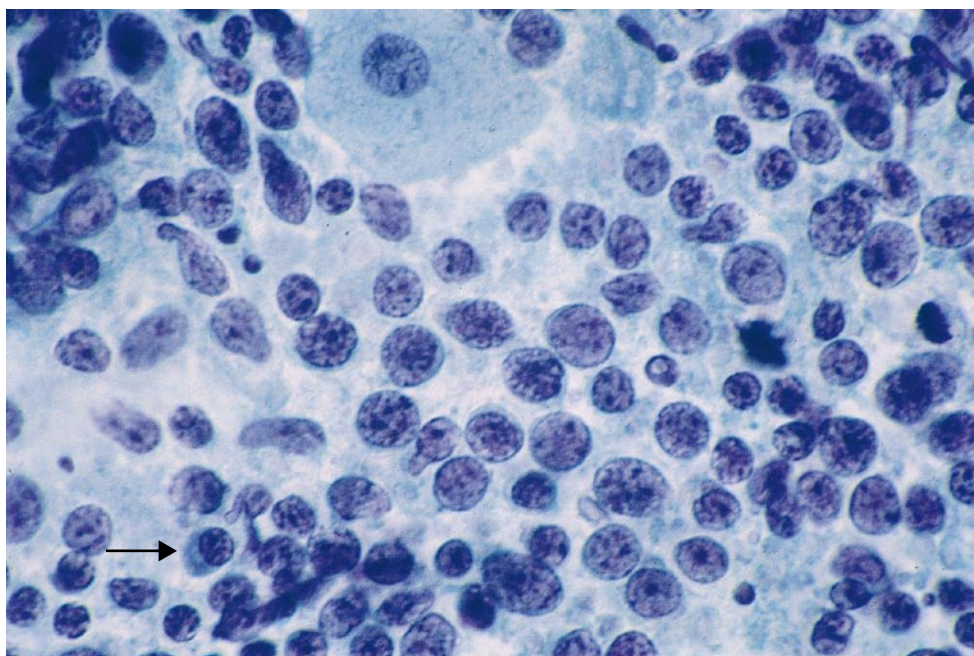


Figure 1.16 Follicular cervicitis. This smear from a 61-year-old woman contains numerous lymphocytes in various stages of maturation, including an occasional plasma cell (arrow). Most normal lymphocytes have a round nuclear contour, unlike the cells of a high-grade squamous intraepithelial lesion (HSIL), to which they bear a superficial resemblance.

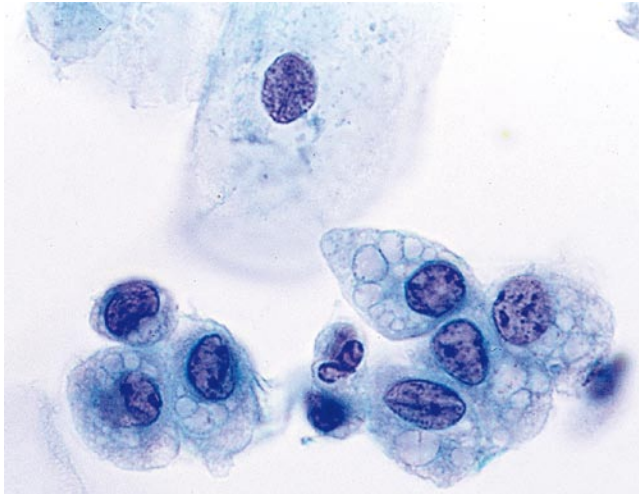


Figure 1.17 Histiocytes. Histiocytes have abundant multivacuolated cytoplasm and an oval, occasionally folded nucleus.

pattern, commonly seen during the second (luteal) phase of the menstrual cycle, is known as cytolysis—bare intermediate cell nuclei, fragments of squamous cytoplasm, and abundant bacterial rods (Fig. 1.18). Cytolysis can interfere with one's ability to evaluate nuclear to cytoplasmic ratio, an important criterion in grading SILs.

Artifacts and Contaminants

The more commonly encountered artifacts and specimen contaminants are illustrated in Figure 1.19.

ORGANISMS AND INFECTIONS

Shift in Flora Suggestive of Bacterial Vaginosis

A steep reduction in the proportion of lactobacilli, with a concomitant predominance of coccobacilli, is associated with bacterial vaginosis, a disorder characterized by a thin, milky vaginal discharge and a foul, fishy odor. At one time attributed solely to *Gardnerella vaginalis*, it is now clear that bacterial vaginosis can be caused by other bacteria as well.¹¹² The diagnosis is made by correlating morphologic findings on a Pap or wet prep with other test results (vaginal pH and the amine-odor “whiff” test after adding potassium hydroxide [KOH]).¹¹³

CYTOMORPHOLOGY OF A SHIFT IN FLORA:

- short bacilli (coccobacilli), curved bacilli, or mixed bacteria
- no lactobacilli
- “filmy” appearance
- “clue cells”

The cytologic hallmark is the replacement of the normal lactobacilli by shorter bacilli (coccobacilli), curved bacilli, and mixed bacteria (Fig. 1.20). These small organisms are numerous and give a filmy appearance to the preparation. They frequently adhere to squamous cells, completely covering them like a shag carpet (“clue cells”). Clue cells are not specific

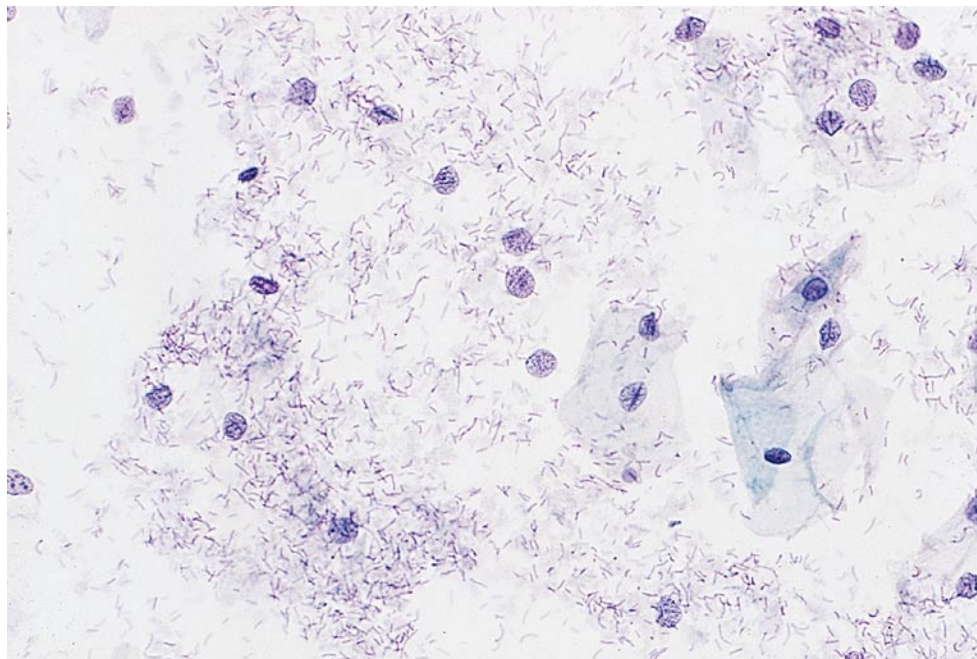


Figure 1.18 Lactobacilli. These bacteria are part of the normal flora of the vagina. Note the bare nuclei of the intermediate cells, which are subject to cytolysis by these organisms.

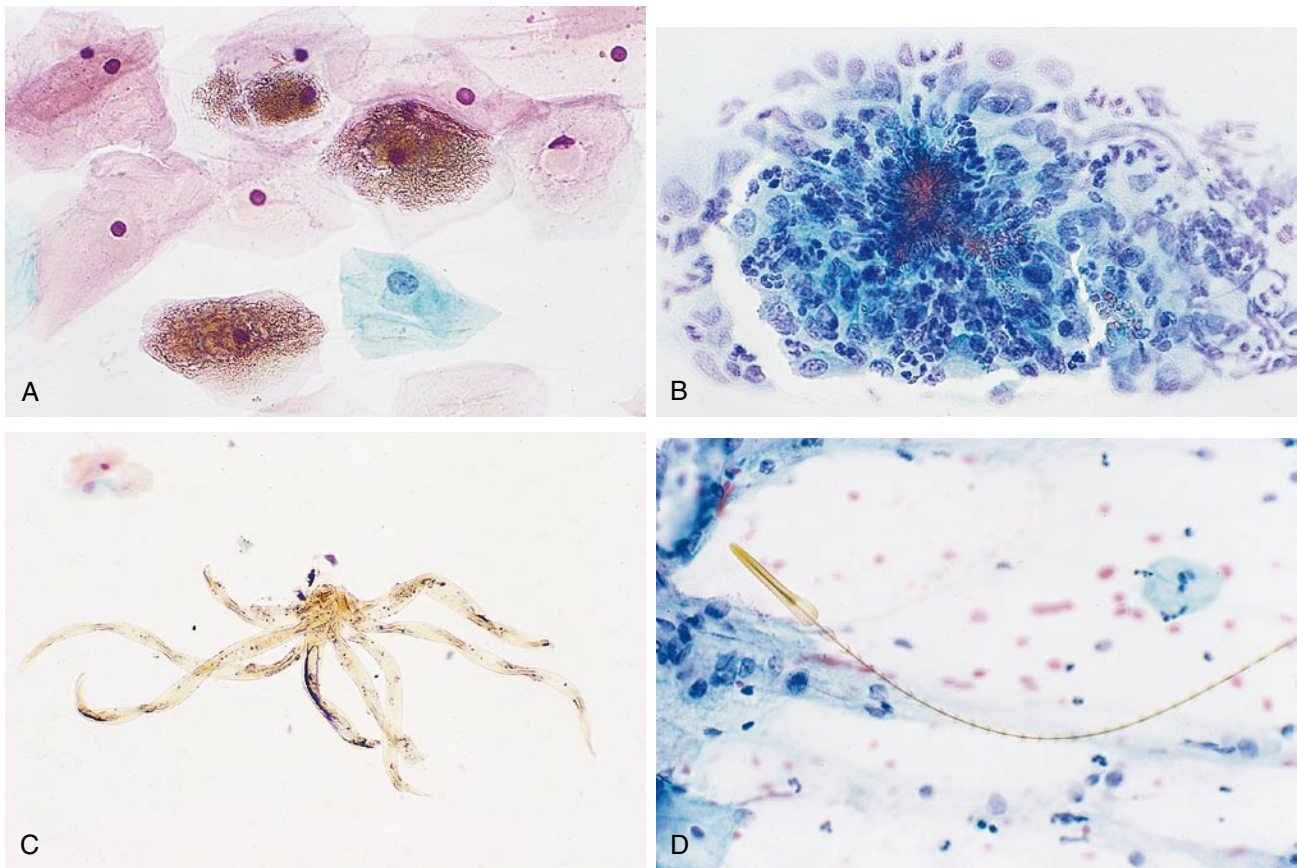


Figure 1.19 Artifacts and contaminants. A, "Cornflaking." This refractile brown artifact results from bubbles of air trapped on superficial squamous cells, resulting in obscuring of the nuclei. It can be reversed by returning the slide through xylene and alcohol to water, then restaining and coverslipping. B, "Cockleburrs." This is the name given to radiate arrays of club-shaped orange bodies composed of lipid, glycoprotein, and calcium, surrounded by histiocytes. They are most commonly associated with, but not limited to, pregnant patients. They have no clinical significance. C, Trichome. These large star-shaped structures are derived from the arrow-wood plant. They stain a pale yellow and have from three to eight legs. Trichomes are produced by many different plants and vary in color, size, and shape. D, Carpet beetle parts. These arrow-shaped structures are contaminants from sources such as gauze pads and tampons.

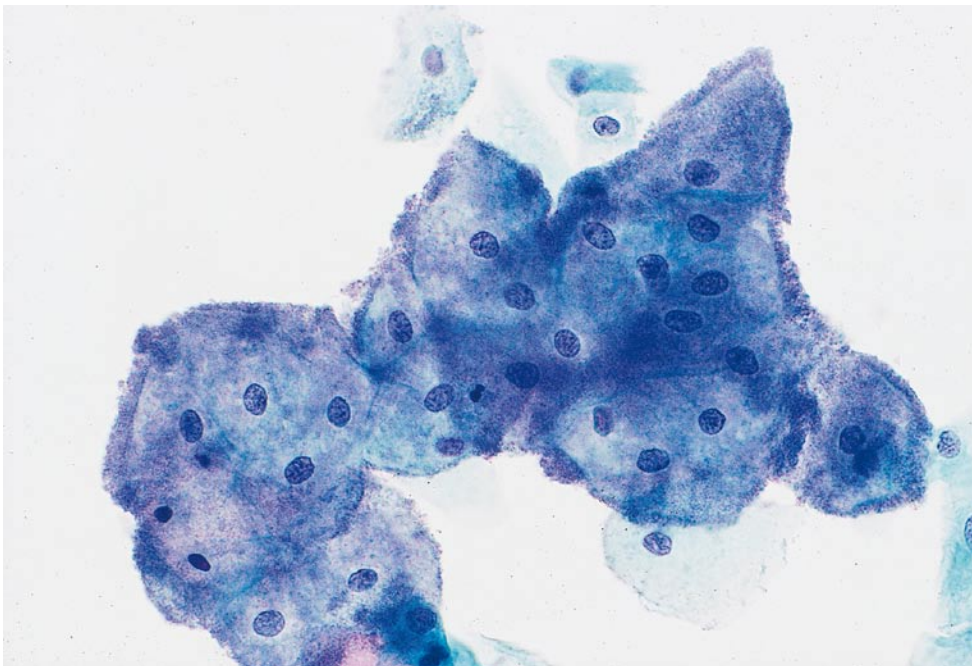


Figure 1.20 Shift in flora suggestive of bacterial vaginosis. Numerous small bacteria cover the slide. In some but not all cases, these bacteria adhere to squamous cells ("clue cells"), giving them the appearance of a shag rug, as seen here. Lactobacilli are absent.

for the diagnosis. Requiring at least 20% clue cells may increase the specificity of the diagnosis.¹¹⁴ Neutrophils are often scarce.

This pattern is common and seen in about 50% of patients referred to a dysplasia clinic.¹¹⁵ Clinical correlation is required for a definite diagnosis of bacterial vaginosis because the cytologic pattern is neither sufficient nor necessary for the diagnosis. Women who are symptomatic are treated with metronidazole or clindamycin.

Trichomonas Vaginalis

Trichomonas vaginalis is a primitive eukaryotic organism, a parasitic protozoan that causes trichomoniasis, a sexually transmitted disease. Patients may experience burning, itching, and a malodorous vaginal discharge, but up to 50% are asymptomatic.¹¹⁶ Although regarded primarily as a disease of women, it also occurs in men, most of whom are asymptomatic.

CYTOMORPHOLOGY OF TRICHOMONAS VAGINALIS:

- 15 to 30 μm long
- pear shaped
- pale, eccentrically placed nucleus
- red cytoplasmic granules

The organism is a 15- to 30- μm pear-shaped protozoan that has a small, pale, eccentrically placed nucleus (Fig. 1.21). The cytoplasm often contains tiny red granules. It is commonly accompanied by *Leptothrix*, a nonpathogenic, long, filamentous bacterium. Some squamous cells have a small, narrow, indistinct perinuclear halo that calls to mind the cytopathic changes

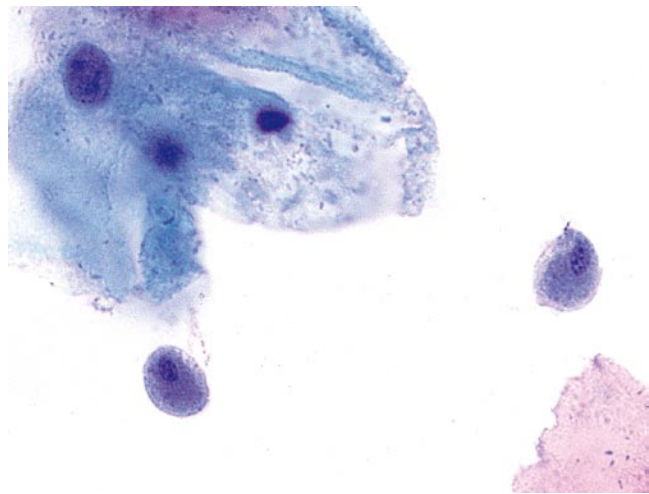


Figure 1.21 *Trichomonas vaginalis*. This organism has an indistinct, ghostly appearance, with a pale oval nucleus and faint red granules.

of HPV, but *Trichomonas*-related halos are smaller and accompanied by only minimal nuclear atypia.

Patients and their sexual partners are treated with metronidazole.¹¹⁶

Candida

Candida albicans and *C. glabrata* are fungal species that infect the vulva, vagina, and cervix. Patients may be asymptomatic, or they may complain of burning, itching, and a thick, cheesy discharge.

CYTOMORPHOLOGY OF CANDIDA:

- pink
- yeast forms (3 to 7 μm diameter)
- long pseudohyphae and true hyphae
- tangles and skewers of squamous cells around pseudohyphae ("spaghetti and meatballs," "shish kebabs")

These fungi are eosinophilic and often interspersed among squamous cells (Fig. 1.22). In many cases, some squamous cells appear in linear arrays, as if skewered by the pseudohyphae. Tangles of pseudohyphae ("spaghetti") admixed with yeast forms ("meatballs") are common. Thin mucus strands are a common mimic of *C. pseudohyphae*, but they are pale blue rather than pink like *Candida*.

Not all women with this finding are symptomatic, and usually only symptomatic women are treated.

Actinomyces

Actinomyces species are gram-positive anaerobic bacteria that are normal inhabitants of the mouth and bowel. They are uncommon in the cervix and vagina, where they are almost always associated with a foreign body, most commonly an IUD. It is estimated that 7% of women with an IUD have *Actinomyces* spp. on their Pap,¹¹⁷ and the frequency is related to the duration of continuous IUD use. When found incidentally on a Pap test, they are almost always harmless. In a small number of cases, however, women with an IUD develop pelvic actinomycosis, usually a tubo-ovarian abscess, presumably as a result of ascending infection. Case reporting has not been systematic, so it is impossible to judge the risk of this significant complication, but pelvic actinomycosis resulting from an IUD is considered exceedingly rare.¹¹⁸

CYTOMORPHOLOGY OF ACTINOMYCES:

- tangled clumps of bacteria ("cotton balls," "dust bunnies")
- long, filamentous organisms
- Figure 1.23

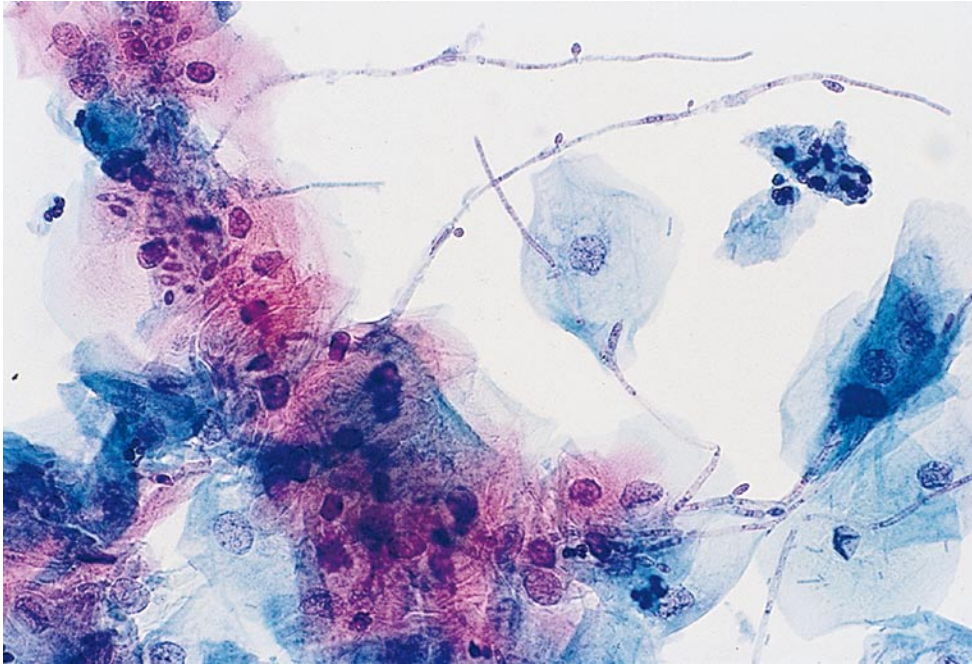


Figure 1.22 *Candida*. Pseudohyphae and yeast forms, some of them budding from pseudohyphae, are seen. Note the skewed squamous cells.

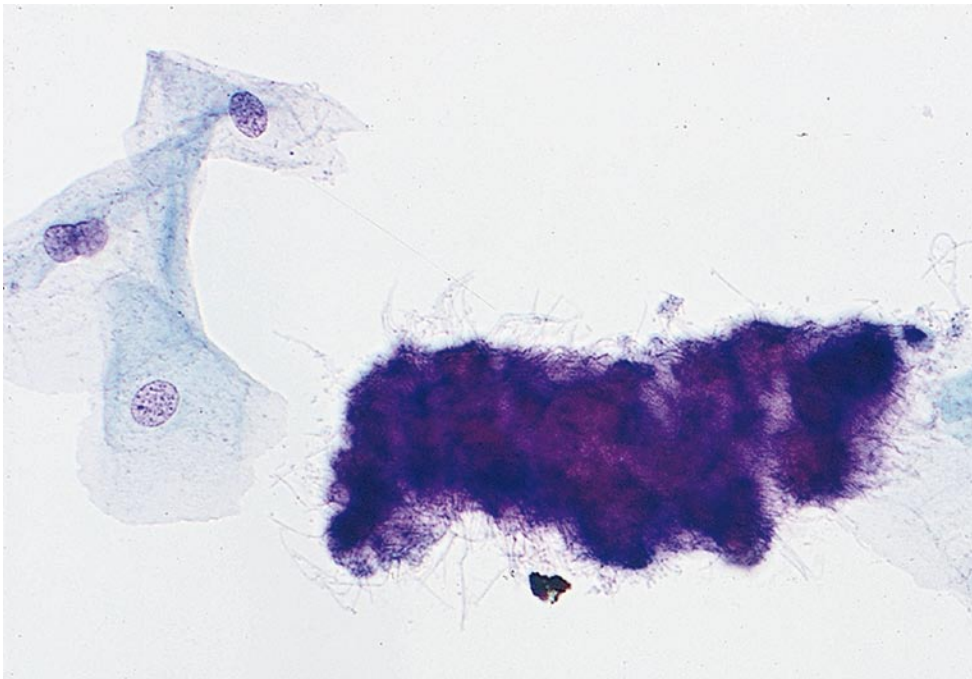


Figure 1.23 *Actinomyces* spp. These bacterial colonies resemble dark cotton balls. The organisms are filamentous, shown here protruding from the mass of bacteria.

If *Actinomyces* are seen on a Pap, removal of the IUD is not necessary, and treatment of asymptomatic women is not recommended.¹¹⁷

Herpes Simplex

Infection by the herpes simplex virus is identified by the characteristic nuclear changes of infected epithelial cells.

CYTOMORPHOLOGY OF HERPES SIMPLEX CYTOPATHIC CHANGES:

- multinucleation
- molding of nuclei
- margination of chromatin
- ground-glass nuclei
- eosinophilic intranuclear inclusions

The nucleus has a homogeneous, glassy appearance (“ground-glass”), and nuclear membranes are thick resulting from peripheral margination of chromatin (Fig. 1.24A). Multinucleation is common, with molding of nuclei. Eosinophilic intranuclear inclusions may be present.

Cytomegalovirus

Exposure to and infection by cytomegalovirus (CMV) is common in the general population, but clinical manifestations, such as mononucleosis, are relatively uncommon. The cytologic changes of cytomegalovirus infection can be seen on cervical-vaginal preparations from women who are immunocompetent and who are immunocom-

promised.¹¹⁹ In patients who are immunocompetent, the infection is transient and usually asymptomatic.

CYTOMORPHOLOGY OF CYTOMEGALOVIRUS CYTOPATHIC CHANGES:

- mononuclear cells
- markedly enlarged
- basophilic intranuclear inclusion
- small granular cytoplasmic inclusions

Infected cells are enlarged, and the nuclei have a solitary basophilic inclusion surrounded by a halo. Multiple small, granular cytoplasmic inclusions are also present (Fig. 1.24B). The infected cells are endocervical or ectocervical in origin.¹²⁰

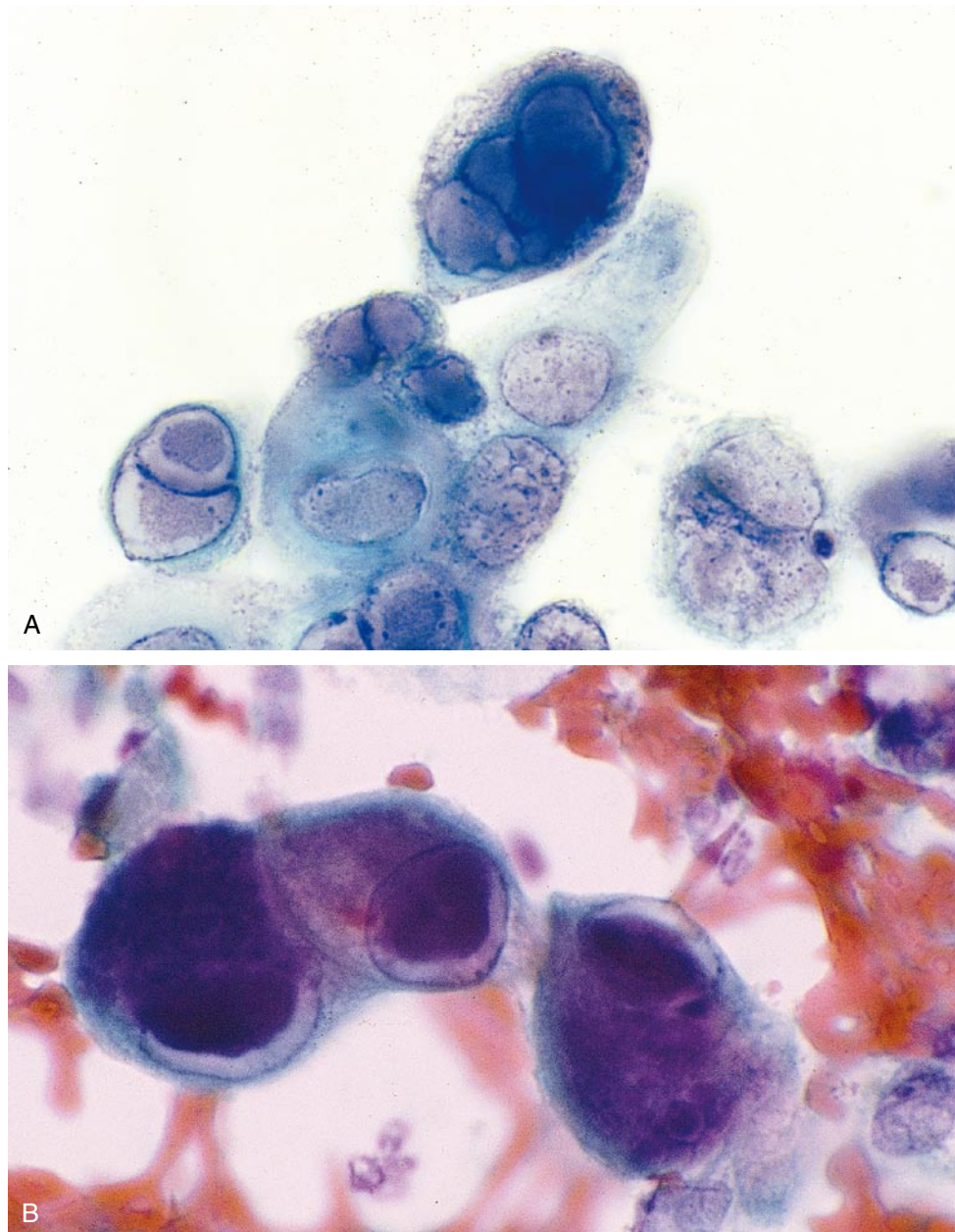


Figure 1.24 Viral cytopathic changes. A, Herpes simplex. The nuclei of infected cells are filled with viral particles, which impart a pale, homogeneous appearance. Nuclear chromatin is visible only at the periphery of some nuclei. Some have a well-defined eosinophilic intranuclear inclusion. B, Cytomegalovirus. Each cell has a large basophilic intranuclear inclusion that is surrounded by a halo; the cytoplasm contains multiple small basophilic inclusions as well. This patient was immunocompetent and asymptomatic, and the inclusions were identified in only a few cells.

Chlamydia Trachomatis

Chlamydia trachomatis is one of the most common sexually transmitted pathogens and a leading cause of cervicitis, endometritis, and pelvic inflammatory disease. Cytologic criteria for diagnosis, such as cytoplasmic vacuolization or an inflammatory infiltrate composed of transformed lymphocytes, have been shown to have low diagnostic accuracy.¹²¹ Laboratories have therefore abandoned cytologic diagnosis in favor of microbiologic testing methods.

Rare Infections

Amebiasis of the female genital tract caused by *Entamoeba histolytica* is uncommon; 10% to 20% of cases have been associated with neoplasms.¹²² The organisms, which range in size from 12 to 40 μm and have a small, eccentric nucleus and abundant vacuolated cytoplasm, may be misinterpreted as large histiocytes. Erythrophagocytosis is common. Unlike *E. histolytica*, *E. gingivalis* is not associated with a pathogenic role in genital infections, although it has been described as accompanying *Actinomyces* spp. in patients using IUDs.¹²³

Granuloma venereum (granuloma inguinale) is a sexually transmitted, ulcerative condition that usually involves the labia, but can cause cervical lesions. The causative organism (*Calymmatobacterium granulomatis*, also known as the Donovan body) is an encapsulated gram-negative bacterium that is concentrated in macrophages and difficult to see with the Papanicolaou stain. A Giemsa stain demonstrates the intracellular organisms.¹²⁴ Another condition in which intracellular bacteria are seen is malakoplakia, which rarely involves the cervix.¹²⁵

BENIGN AND REACTIVE CHANGES

Trauma, infections, hormonal stimulation, radiation, and other factors cause a variety of morphologic alterations of squamous and endocervical cells that range from the mild to the alarmingly exuberant. At their most extreme, reactive epithelial changes mimic malignancy. For this reason, federal regulations require that a cytotechnologist refer all cases with “reactive or reparative” changes to a pathologist for review (see Chapter 17). Because the word *reactive* is rather nebulous, defining precisely which morphologic alterations require pathologist review is up to the laboratory director, and implementation rests on the judgment of the cytotechnologist. Thus, familiarity with the characteristic morphology of reactive changes is important and helps prevent misdiagnosis. Inflammatory changes affect both squamous and endocervical cells, but the changes are often more dramatic in endocervical cells.

Benign Squamous Changes

Mature squamous cells can show a variety of nuclear and cytoplasmic changes, most commonly *simple nuclear enlargement* of intermediate squamous cells without hyperchromasia or nuclear membrane irregularity. The nuclear enlargement is usually slight (one-and-a-half to two times the area of a normal intermediate cell nucleus), but sometimes is greater. Despite the nuclear size increase, the chromatin is finely and uniformly granular. Bland nuclear enlargement of intermediate cells is particularly common in Pap samples from perimenopausal women (aged 40 to 55 years). Because of this association they have been termed *PM* (for perimenopausal) *cells* (Fig. 1.25). Without accompanying hyperchromasia or

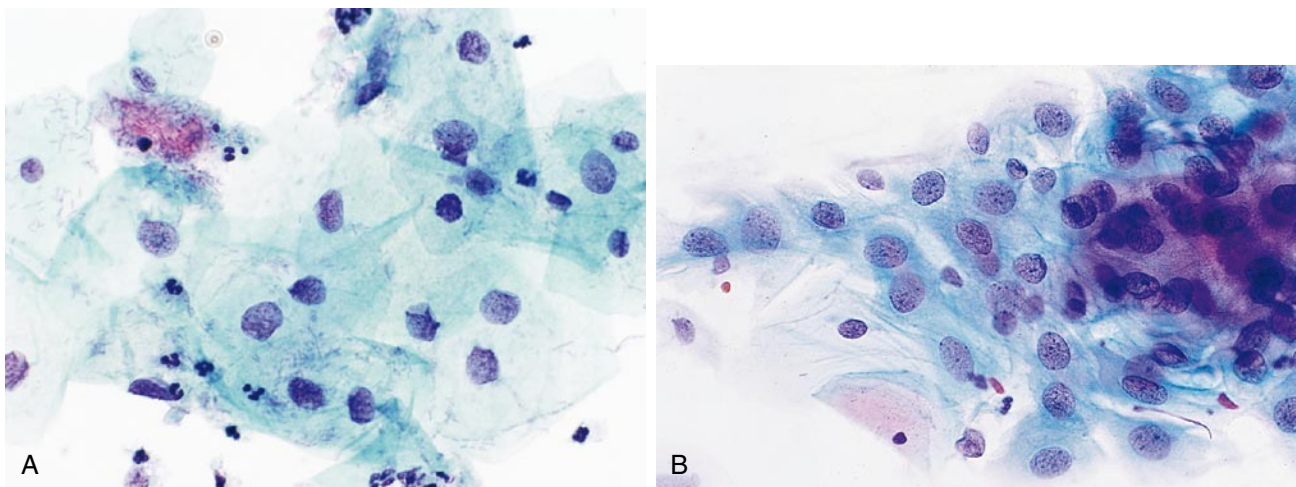


Figure 1.25 Benign squamous cell changes. A, PM cells. Nuclear enlargement, with little in the way of nuclear membrane irregularity or hyperchromasia, is a common finding in intermediate squamous cells from perimenopausal women. Such bland nuclear enlargement should not be mistaken for a significant atypia. B, A similar bland nuclear enlargement occurs in metaplastic cells.

nuclear membrane irregularity, these cells are unlikely to represent a significant squamous lesion.¹²⁶ The cause of nuclear enlargement in squamous cells from perimenopausal women is not known.

Nonspecific perinuclear cytoplasmic clearing in superficial and intermediate squamous cells is associated with inflammatory conditions like *Trichomonas* infection, but it can also be a slide preparation artifact. It is distinguished from koilocytosis by the small size of the halo and the absence of increased cytoplasmic density outlining the cavity (Fig. 1.26A). Large cytoplasmic clearings occur in squamous cells with abundant cytoplasmic glycogen. They are distinguished from LSIL cells because they have a normal intermediate cell nucleus (Fig. 1.26B).

Squamous metaplastic cells are particularly prone to reactive changes. There can be nuclear enlargement and

variation in nuclear size, and nucleoli are sometimes prominent. Smooth nuclear membranes and finely textured chromatin are reassuring. In some cases, however, the alterations in metaplastic squamous cells are more marked and overlap with the features of HSIL. Such borderline cases are called atypical squamous metaplasia.

Benign Endocervical Changes

Reactive endocervical cells often show much greater increases in nuclear size than squamous cells. Some reactive endocervical cell nuclei are four or five times larger than normal, usually with an accompanying increase in cytoplasm. The enlarged nuclei remain round or oval, but they frequently have a large nucleolus (Fig. 1.27). Such changes are not uncommon in pregnancy, where in

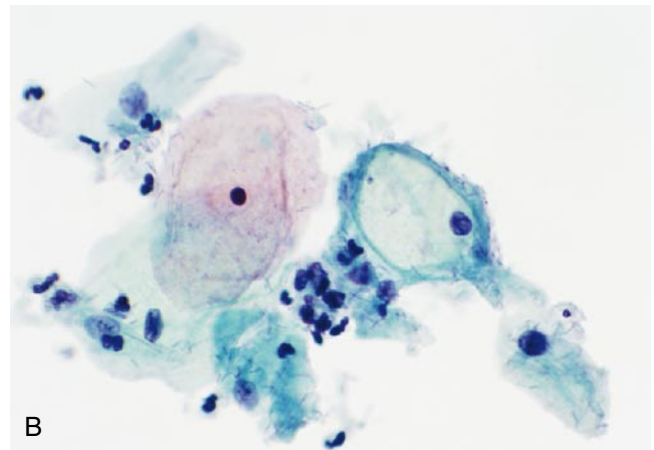
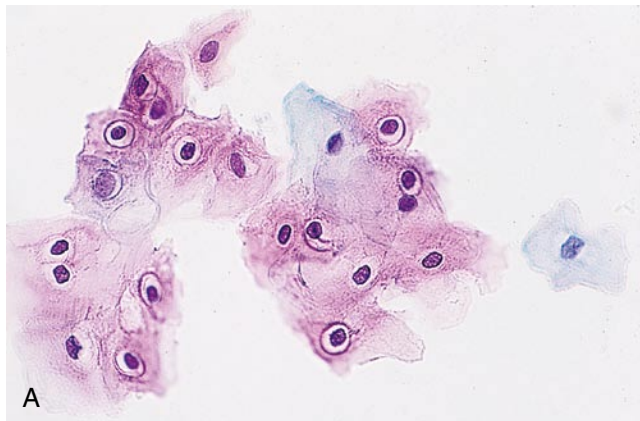


Figure 1.26 Nonspecific halos. A, Small halos around the nuclei of squamous cells are nonspecific and do not represent human papillomavirus (HPV)-related changes. B, Some normal squamous cells have abundant glycogen that mimics koilocytosis. Note the normal nucleus.

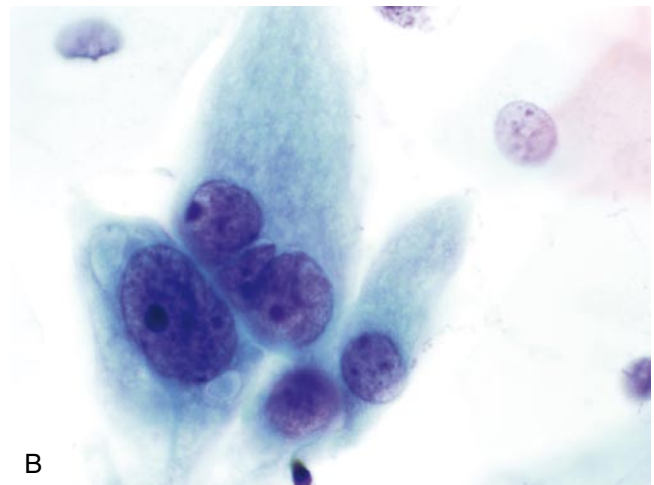
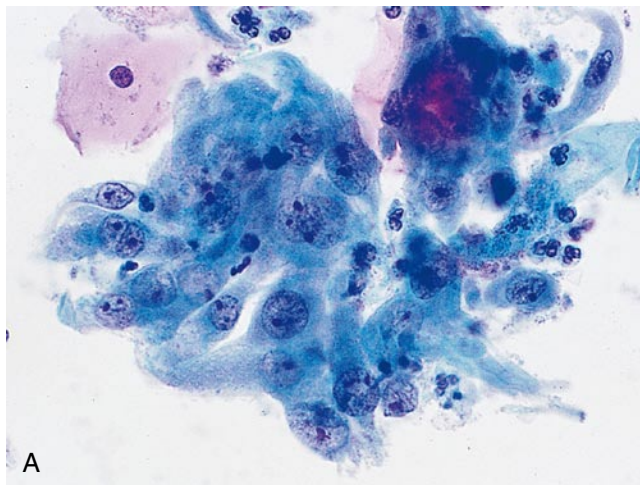


Figure 1.27 Reactive endocervical cells. A, A common finding, reactive endocervical cells are enlarged and have a prominent nucleolus. B, Isolated cells can be as big as mature squamous cells and mimic a low-grade squamous intraepithelial lesion (LSIL), but a prominent nucleolus is uncharacteristic of an LSIL.

their extreme form they represent the Arias-Stella reaction.¹²⁷ They are also seen in patients with endocervical polyps and inflammation of any cause.

Reactive endocervical cells are also seen in *microglandular hyperplasia*, a benign alteration of endocervical epithelium associated with oral contraceptive use. Microglandular hyperplasia was originally described in histologic material, where it was sometimes confused with adenocarcinoma. Cytologic changes range from entirely normal endocervical cells to marked nuclear enlargement, often with prominent nucleoli and cytoplasmic vacuolization (Fig. 1.28).¹²⁸ Clinical correlation is useful. Knowledge that the patient is pregnant or has a visible endocervical polyp can alert the cytologist to the possibility of reactive changes and provide a rational explanation for the alterations. In their most extreme forms, however, reactive endocervical cells raise a differential diagnosis that includes LSIL, HSIL, AIS, and

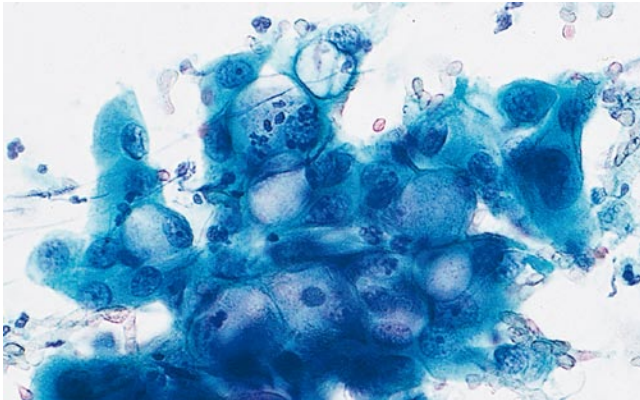


Figure 1.28 Reactive endocervical cells (microglandular hyperplasia). These cells are enlarged and have a prominent large cytoplasmic vacuole.

invasive cancer. The differential diagnosis of reactive endocervical cells is discussed in greater detail in the corresponding sections that follow. Ultimately, the benign nature of reactive endocervical cells is betrayed by the roundness of the nucleus, its fine chromatin granularity, and the normal nuclear-to-cytoplasmic ratio.

Repair

Reparative changes result from injury to the cervical epithelium and the proliferation of reserve cells, which grow to reepithelialize a focus of ulceration.

CYTOMORPHOLOGY OF REPAIR:

- cohesive, flat sheets
- streaming appearance
- large nucleus with marked size variation
- large nucleolus, sometimes irregular
- pale chromatin
- mitoses

Typical repair is composed of flat sheets of cells that have an enlarged nucleus, a prominent nucleolus, and occasional mitoses. Repair cells often maintain a uniform polarity that gives the sheets the appearance of streaming (like a school of fish) or being pulled out (like taffy; Fig. 1.29). Because the sheets are cohesive, individual abnormal cells—so characteristic of carcinomas—are generally absent in repair reactions. Nevertheless, some repair reactions are so extensive, with unusual features, such as crowded nuclei and a coarsely granular chromatin texture, that doubt about their benign nature is raised. Such a case is best interpreted as “atypical squamous cells, with features of atypical repair.”

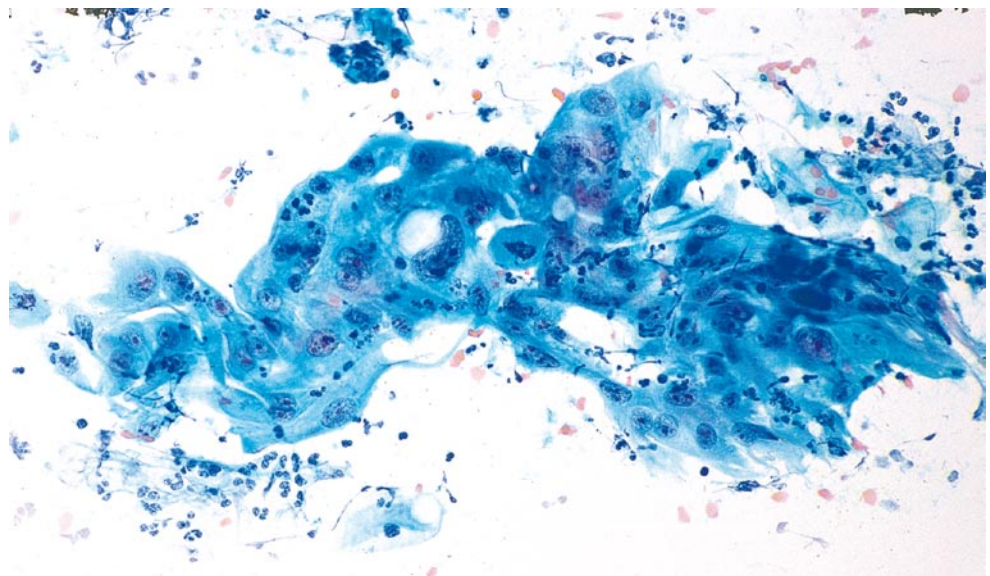


Figure 1.29 Typical repair. Reparative epithelium is cohesive and arranged in a monolayered, streaming sheet.

DIFFERENTIAL DIAGNOSIS OF REPAIR:

- nonkeratinizing SQC (see Fig. 1.47)
- endocervical adenocarcinoma (see Fig. 1.62)

Reparative epithelium does not resemble LSIL, HSIL, or AIS. Rather, it leapfrogs over precursor lesions and audaciously mimics invasive cervical cancers (e.g., nonkeratinizing SQC and adenocarcinoma). The resemblance stems from the combination of large round nuclei, prominent nucleoli, and mitoses. However, the distinction from cancer is usually straightforward. Reparative epithelium may be associated with inflammation, but the necrotic debris typical of invasive cancers is absent. Invasive cancers often contain sheets and clusters of malignant cells, but there are usually numerous isolated malignant cells as well, whereas reparative epithelial cells are famously cohesive. Nonkeratinizing SQCs have coarsely textured chromatin rather than the fine granularity of repair cells.

Radiation Changes

Radiation induces changes in cells that either disappear with time or persist for many years.

CYTOMORPHOLOGY OF RADIATION CHANGES:

- large, bizarre cells
- normal nuclear-to-cytoplasmic ratio
- cytoplasmic vacuolization and polychromasia
- multinucleation

The characteristic changes are marked cellular and nuclear enlargement with preservation of the nuclear-to-cytoplasmic ratio, cytoplasmic vacuolization, and cytoplasmic polychromasia (“two-tone” cytoplasm) (Fig. 1.30). Nuclei have finely granular chromatin or show smudgy hyperchromasia, and there can be nuclear and cytoplasmic vacuolization. Cells are isolated or arranged in groups, and multinucleation is common. Reparative epithelium commonly accompanies radiation changes. Some chemotherapeutic drugs induce similar changes.

DIFFERENTIAL DIAGNOSIS OF RADIATION CHANGES:

- herpes cytopathic changes
- recurrent cancer
- LSIL

Radiation changes superficially resemble herpes cytopathic changes. Multinucleation occurs in both conditions, but radiation lacks the ground glass nuclear appearance or Cowdry A type inclusions typical of herpes. If the radiation was given for a cervical cancer, the differential diagnosis includes recurrent SQC or adenocarcinoma of the cervix, with superimposed radiation changes. The cells of a recurrent SQC and adenocarcinoma are typically more numerous than the scattered radiation cells. Recurrent cancers show more significant nuclear atypia than is seen in radiation. Coarsely textured chromatin (rather than smudgy hyperchromasia) is typical of nonkeratinizing SQC.

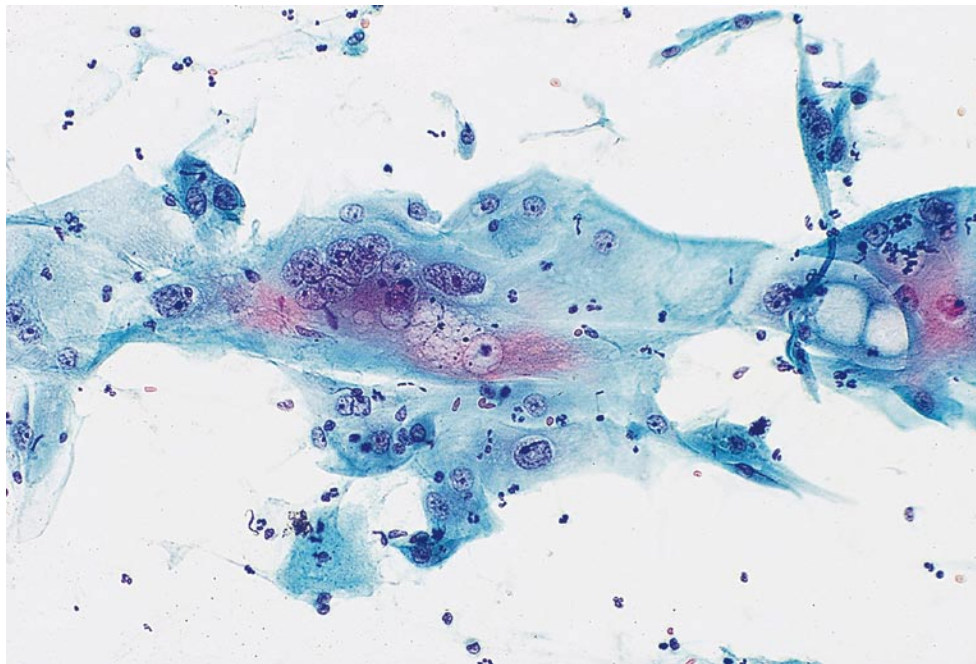


Figure 1.30 Radiation effect. Radiation looks like a wild reparative reaction, with large cells, multinucleation, cytoplasmic vacuolization, and a curious “two-tone” cytoplasmic staining pattern.

Cellular Changes Associated with Intrauterine Devices

There are two distinct cell types that are associated with IUD use.¹²⁹

CYTOMORPHOLOGY OF INTRAUTERINE DEVICE EFFECT:

- vacuolated cells
- small dark cells with scant cytoplasm
- Figure 1.31

The first type of “IUD cell” is a glandular cell that is arranged in small groups (5 to 15 cells) or as isolated cells. It has abundant vacuolated cytoplasm, and in some cells a large vacuole displaces the nucleus. Nuclei are enlarged and nucleoli are usually visible. The second pattern consists of isolated small cells of uncertain histogenesis. They have a hyperchromatic nucleus and a high nuclear-to-cytoplasmic ratio. Sometimes reparative changes are also present and the background is inflamed.

DIFFERENTIAL DIAGNOSIS OF INTRAUTERINE DEVICE EFFECT:

- adenocarcinoma
- HSIL

The vacuolated cells of IUD effect are virtually indistinguishable from the cells of an adenocarcinoma, particularly those of endometrial origin. If the woman has an IUD, these changes are most likely benign, but clinical correlation and a repeat Pap after removal of the IUD might be considered. The small IUD cells resemble HSIL cells except that they have a nucleolus.¹³⁰

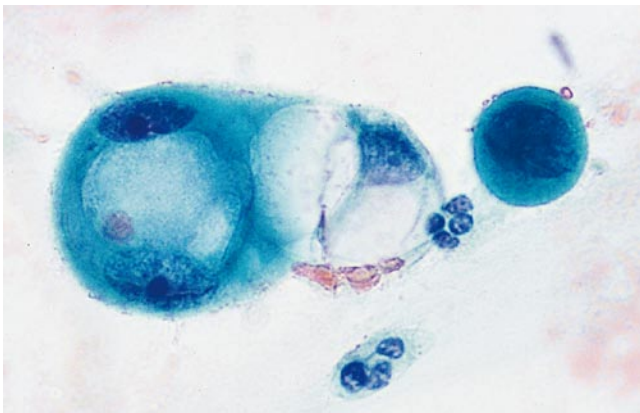


Figure 1.31 Intrauterine device (IUD) effect. The two types of cells are seen here: a vacuolated cell and a small dark cell with scant cytoplasm. This combination is characteristic of IUD effect.

Glandular Cells Status Post Hysterectomy

Glandular cells resembling normal endocervical cells are seen in approximately 2% of vaginal Pap samples from women who have had a total hysterectomy.¹³¹ This finding is more common in women who have had postoperative radiotherapy and may therefore represent a therapy-induced metaplasia of squamous epithelium. If they resemble normal endocervical cells, they are entirely benign (Fig. 1.32) and need not raise the possibility of an adenocarcinoma, even if the hysterectomy was carried out for an adenocarcinoma of the cervix or endometrium. A line in the report noting “Benign glandular cells status post hysterectomy” is appropriate.

Given that some hysterectomies are supracervical, sometimes endocervical cells on a vaginal Pap from a woman who has had a hysterectomy are truly cells from the cervical stump. Careful review of the operative notes can help clarify this possibility.

Other Benign Changes

The cells of tubal metaplasia of the endocervix often look like normal endocervical cells, except that they have cilia. Sometimes they have a higher nuclear-to-cytoplasmic ratio and slight hyperchromasia and may be mistaken for a significant squamous or glandular lesion if a careful search is not made for cilia.¹³² Cilia are reliable evidence that the cell they are attached to is benign because ciliated adenocarcinomas of the endocervix are uncommon.^{133,134} Endometriosis of the cervix resembles abraded endometrium (see “Abraded endometrium and lower uterine segment” above).

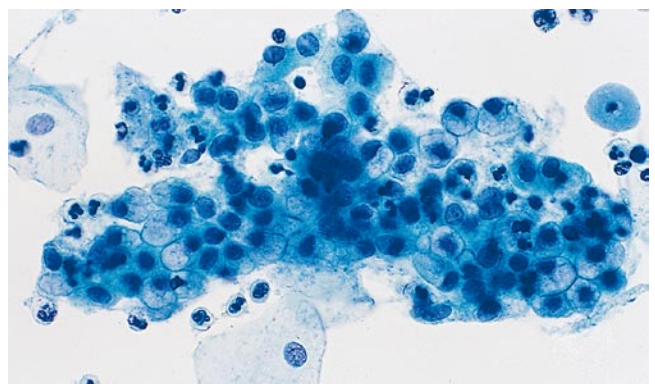


Figure 1.32 Glandular cells status posthysterectomy. The squamous mucosa of the vagina has undergone mucinous metaplasia.

VAGINAL SPECIMENS IN “DES DAUGHTERS”

The daughters of women who were given DES during pregnancy to prevent a threatened abortion are at risk for a variety of abnormalities, most of them benign, of the vagina, cervix, and uterus. About one third of these DES daughters develop vaginal adenosis, the presence of glands in the vagina. Mucinous epithelium is the most frequently encountered type of glandular epithelium, but tubal and endometrial-type epithelia are sometimes seen. A diagnosis of vaginal adenosis is supported by the presence of glandular or squamous metaplastic cells on a direct sample from the wall of the vagina.

Clear cell carcinoma of the vagina is the least common but most dreaded complication of in-utero DES exposure.

SQUAMOUS ABNORMALITIES

Squamous Intraepithelial Lesions

The term *squamous intraepithelial lesion* encompasses the spectrum of precursors to invasive SQC, previously called “dysplasia,” “carcinoma in situ,” “borderline lesion,” and “CIN.” Strong evidence links SIL with invasive squamous cancer. Epidemiologic risk factors (e.g., sexual history) are similar for patients with SIL and those with invasive cancer, and both are associated with HPV. Both SIL and cancer have similar chromosomal abnormalities as measured by cytogenetic or image analysis methods. Women with SIL are at least 10 years younger on average than those with invasive cancer; this chronology suggests progression of SIL to invasion. Finally, SIL resembles cancer morphologically and is often present in histologic sections directly adjacent to invasive cancer.

The natural history of SIL is not easy to study. Ethical considerations prohibit using a control group, especially women with high-grade lesions.¹³⁵ Many studies have chosen a high-grade lesion as their endpoint for investigating the behavior of low-grade lesions because allowing a lesion to progress to invasive cancer is out of the

question. Yet it is precisely the risk of progression to invasion that is of paramount interest. A biopsy itself interferes with the natural history of a lesion by removing it entirely or by causing a surrounding inflammatory reaction that can destroy it.¹³⁶ Follow-up biopsies or smears may not be representative of the underlying lesion, and followup time may be inadequate. Finally, the criteria for diagnosing and grading SIL differ among observers. A meta-analysis of this large and heterogeneous body of data suggests that about 50% of LSILs regress, and only about 0.15% progress to invasive cancer in 2 years.⁸³ Fewer HSILs regress, and many more progress to invasive cancer (Table 1.3).

The sexually transmitted HPV explains the well-known epidemiologic association between sexual history and increased risk of cervical cancer. Although detected in virtually all cervical cancers by current molecular techniques,¹³⁷ HPV was originally identified in association with a distinctive altered squamous cell known as a *koilocyte*. This unusual cell was first described in 1949 by Ayre, who called it “precancer cell complex,” speculating that it was a precursor to cancer.¹³⁸ In 1960 he correctly suggested a viral etiology. They were recognized by Papanicolaou, who illustrated them with “dyskaryotic” cells in his *Atlas of Exfoliative Cytology*.¹³⁹ The term *koilocytosis* was coined by Koss and Durfee in 1956 after the Greek *koilos* (“hollow”) because of the prominent, sharply defined cytoplasmic cavities of the cells.¹⁴⁰ Two decades later, two groups of investigators working independently made the connection between koilocytes and HPV.^{141,142} Subsequent ultrastructural,¹⁴³ immunocytochemical, and in-situ hybridization¹⁴⁴ studies confirmed the presence of virus within koilocytes (Fig. 1.33). When it was first realized that these changes were the result of a virus, an attempt was made to separate them from dysplasia and CIN.¹⁴¹ Ultimately, it became apparent that a morphologic distinction was not possible,¹⁴⁵ and evidence began to accumulate linking HPV to the pathogenesis of squamous cancer.^{146–148} Currently there is little doubt that HPV plays a central role in causing cervical cancer.

The small HPV genome consists of about 8000 base pairs of circular double-stranded DNA. It codes for only eight genes (Fig. 1.33), which are classified as “early” (E) or “late” (L) depending on the timing of their expression in

TABLE 1.3 THE NATURAL HISTORY OF CYTOLOGIC PREINVASIVE SQUAMOUS LESIONS (FOLLOWUP AT 24 MONTHS)

	Regress (%)	Progress to HSIL (%)	Progress to invasive cancer (%)
ASC-US	68	7	0.25
LSIL	47	21	0.15
HSIL	35	—	1.4

ASC-US, atypical squamous cells of undetermined significance; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion. From Melnikow J, Nuovo J, Willan AR, et al.: Natural history of cervical squamous intraepithelial lesions: A meta-analysis. *Obstet Gynecol* 1998;92(4 Pt 2):727-735.

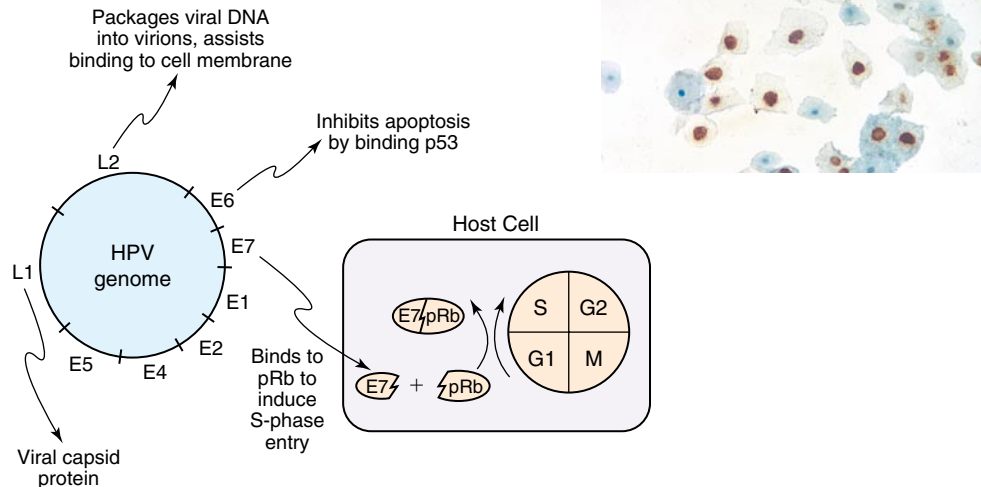


Figure 1.33 The human papillomavirus (HPV) genome and its effects on the host cell. The HPV genome has early (E) and late (L) genes. The E6 and E7 genes are most responsible for the transforming effects of integrated HPV DNA on the host cell. *Inset:* Detection of HPV by in situ hybridization. The dark brown signal is centered on the nucleus of infected cells. (Courtesy of Miu-Fun Chau, DakoCytomation, Carpinteria, Calif.)

the epithelium. HPV infection is established in the basal layers of the epithelium, where the HPV genome is maintained, with expression of the E genes. As the epithelium matures toward the surface, gene amplification and viral assembly occur, with expression of L1 and L2, with eventual viral release. L1 is the major viral capsid protein and is the principal component of the HPV vaccines. The E6 and E7 gene products play the most significant part in cervical oncogenesis. They have a number of cellular targets, with a multitude of effects that lead to malignant transformation.¹⁴⁹ The two most important appear to be (1) the binding of E6 to p53, which results in the blocking of apoptosis, and (2) the binding of E7 to the retinoblastoma tumor suppression protein pRb, which abolishes cell-cycle arrest and leads to unscheduled cellular proliferation.^{149,150}

More than 100 types of HPV have been isolated, of which more than 40 infect the female genital tract. Only a minority cause cervical cancer. The genital HPVs are divided into low-risk and high-risk types based on the frequency of their association with invasive cervical cancer. By definition, an HPV is low risk if it has never been isolated from a cervical carcinoma and high risk if it ever has been. Persistent infection with any one of about 15 high-risk (carcinogenic) types accounts for virtually all cervical cancers.¹⁴⁹

Examples of low-risk and high-risk human papillomaviruses:

- low-risk: 6, 11, 42, 43, 44, 53, 54, 57, and 66
- high-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68

HPV 16 is the prototype of the high-risk viruses and the one most commonly detected in cervical cancers.

A variety of molecular techniques—the polymerase chain reaction, in situ hybridization (Fig. 1.33 inset), and hybrid capture—can be used to detect HPV within cervical lesions. The Hybrid Capture 2™ test, which was evaluated in the multicenter ASCUS/LSIL Triage Study (ALTS) trial sponsored by the National Cancer Institute, uses a cocktail of probes to the 13 high-risk HPV types listed, which account for nearly 90% of HPVs detected in HSIL and invasive cancers.³⁸

The risk of HPV infection per sexual contact is not known but is probably fairly high. Most women, if they are sexually active, are infected with one or more HPV types at some point in their lives. For unclear reasons, the virus has a strong predilection for the transformation zone. Serology is not an accurate measure of infection, because only 50% to 60% of infected women have circulating antibodies to HPV.¹⁵¹ Clearly, only a minority of HPV infections persist and lead to cancer. Cellular immune responses play a role in clearing infection, but how they work is still poorly understood.

Grading Squamous Intraepithelial Lesions

The Bethesda System recommends a low-grade/high-grade approach to grading SIL. This is based on the evidence that most LSILs are transient infections that carry little risk for oncogenesis, whereas most HSILs are associated with viral persistence and a significant potential for progression to invasive cancer.

LSIL encompasses lesions previously described separately as koilocytosis (flat condyloma) and mild dysplasia (CIN 1). The distinction between condyloma and CIN 1 is not reproducible,^{152,153} and both lesions contain a heterogeneous distribution of low-risk and high-risk HPV types. HSIL encompasses lesions previously described as moderate dysplasia (CIN 2) and severe dysplasia or carcinoma in situ (CIN 3). HPV typing plays no role in the grading of SIL. Although low-risk viruses are more common in LSIL than HSIL, high-risk viruses predominate in both.^{38,154} Morphologic assessment by conventional light microscopy is still the gold standard for grading SILs.

Low-Grade Squamous Intraepithelial Lesion

LSIL is a low-risk intraepithelial lesion that is encountered in approximately 2% of all Pap samples.⁸⁷ LSIL is caused by a large number of different HPVs, including low-risk and high-risk types. Many LSILs regress spontaneously (Table 1.3), but some persist for long periods of time. Approximately 21% progress to HSIL, but it is possible that at least some of these may have been HSILs from the beginning but were initially misclassified as LSILs. In fact, 18% of women with an LSIL Pap result prove to have HSIL (CIN 2,3) on biopsy.¹⁵⁵ Less than 1% of untreated LSILs progress to invasive cancer.⁸³

CYTOMORPHOLOGY OF LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESION:

- intermediate-sized cells
- nuclear atypia:
 - enlargement
 - irregular contour
 - hyperchromasia
 - slight chromatin coarseness
- cytoplasmic cavities (koilocytes)
- keratinizing variant

LSIL is a lesion of intermediate or superficial cells that shows nuclear enlargement accompanied by moderate variation in nuclear size and slight irregularities in nuclear shape and contour. Hyperchromasia is present and can take the form of either a uniformly granular increase in chromatin or the smudgy hyperchromasia seen in some koilocytes. Nucleoli are inconspicuous. Classic koilocytes have large, sharply defined perinuclear cytoplasmic cavities surrounded by dense rims of cytoplasm. Their nuclei are usually enlarged and atypical, but not always, and they are diagnostic of LSIL even in the absence of nuclear enlargement (Fig. 1.34). Some LSILs show prominent keratinization manifested by deeply orangeophilic cytoplasm and squamous pearls (Fig. 1.35).

DIFFERENTIAL DIAGNOSIS OF LOW-GRADE SQUAMOUS INTRAEPITHELIAL LESION:

- reactive squamous cells
- squamous cells with nonspecific halos
- reactive endocervical cells
- ASC-US

Nuclear enlargement by itself is not diagnostic of LSIL. It is common with benign squamous cells, particularly those seen in perimenopausal women (PM cells; see Fig. 1.25A). Similarly, small, nonspecific halos mimic the cavities of koilocytes. They are seen in association with *Trichomonas* and other infections, and they can be an artifact of slide preparation. Nonspecific halos are often smaller than koilocyte cavities (see Fig. 1.26A) and unassociated with nuclear atypia (see Fig. 1.26B). Some markedly enlarged reactive endocervical cells have the size and polygonal shape of a squamous cell. With their enlarged nucleus they mimic an LSIL (see Fig. 1.27B). They are recognized by the company they keep (arranged alongside smaller, more recognizable endocervical cells) and by their granular rather than smooth cytoplasm.

Mild but noticeable nuclear changes and larger cytoplasmic cavities raise the possibility of LSIL but sometimes fall short qualitatively or quantitatively. Squamous cells that are suspicious but not conclusive for LSIL are reported as ASC-US.

The management of a woman with an LSIL Pap depends on her particular circumstances. Except for adolescents and postmenopausal women, colposcopy is recommended³⁹ (Fig. 1.36). If the patient is pregnant, it is acceptable but not necessary to defer colposcopy until 6 weeks postpartum. If the patient is not pregnant, the addition of endocervical sampling is acceptable and is in fact preferred when colposcopy is unsatisfactory or when no lesion is seen. HPV testing to women in triage with LSIL Pap samples is not recommended because the high rate of positivity (83%) limits its usefulness.¹⁵⁴ If colposcopy reveals a histologic CIN 2,3, the lesion is surgically excised or ablated.¹⁵⁶ If colposcopy does not reveal CIN 2,3, one has the option of repeating the Pap at 6 and 12 months or performing an HPV test at 12 months. If the HPV test is positive or if either of the repeat Pap tests shows ASC-US or greater, colposcopy is indicated. If the HPV test is negative or the two repeat Pap tests are negative, a return to routine Pap screening is recommended. The routine use of diagnostic excisional procedures like loop electrosurgical excision procedure (LEEP) or ablative procedures is unacceptable in the absence of a biopsy-proven CIN.

Adolescents with LSIL show high rates of lesion regression. For this reason, follow-up with annual Pap testing rather than colposcopy is recommended.³⁹ At the 12-month follow-up, only adolescents with a Pap showing HSIL or greater should be referred to

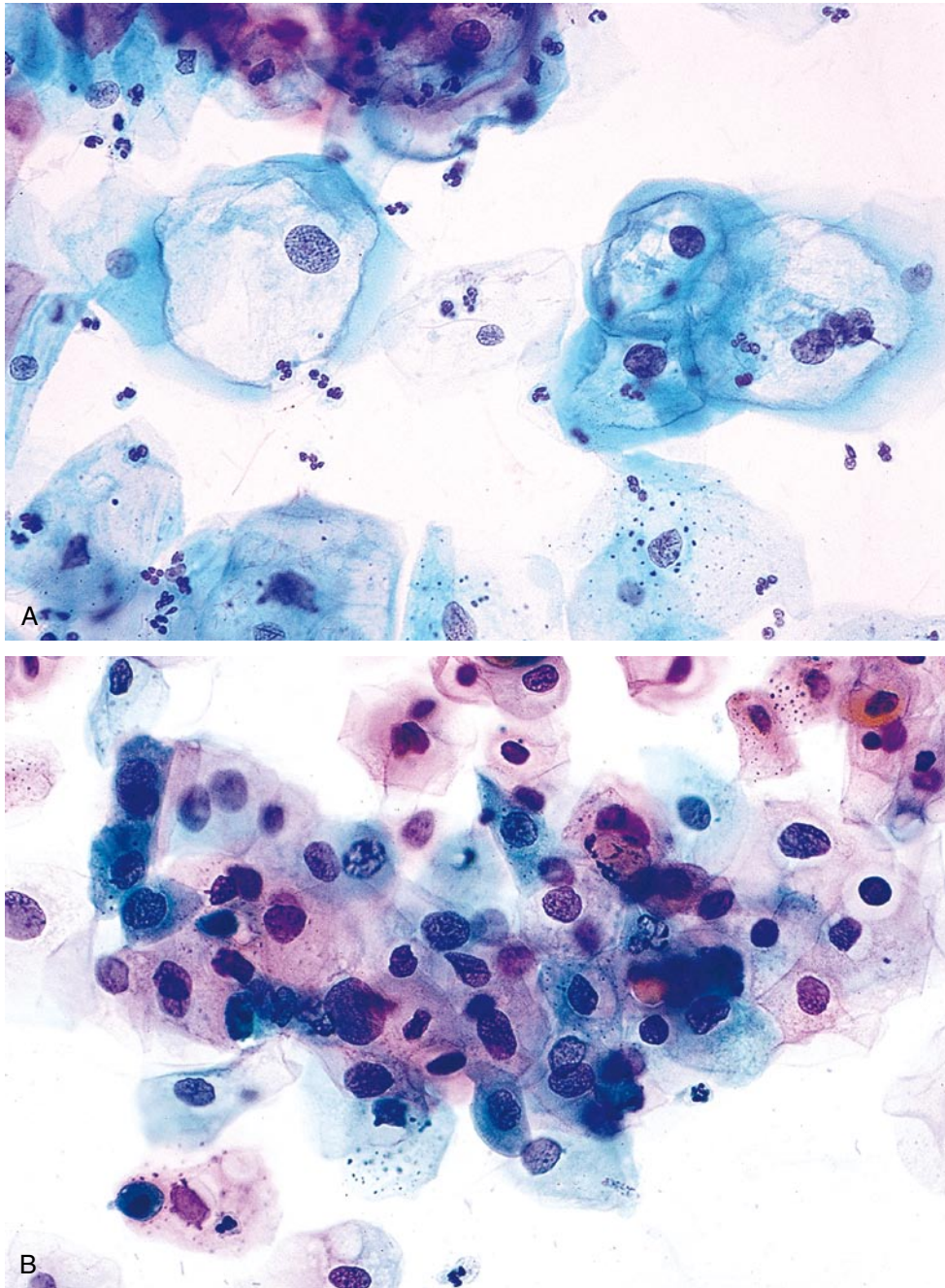


Figure 1.34 Low-grade squamous intraepithelial lesions (LSIL). A, LSIL. Classic koilocytes, as seen here, have a large cytoplasmic cavity with a sharply defined inner edge and are frequently binucleated. Nuclear enlargement may not be as marked as in the nonkoilocytic LSILs. B, Nonkoilocytic LSIL. Nuclei are significantly enlarged and show mild hyperchromasia and nuclear contour irregularity. No definite koilocytes are seen. This pattern was once called mild dysplasia or CIN 1.

colposcopy. At the 24-month follow-up, those with a Pap showing ASC-US or greater should be referred for colposcopy.

As with adolescents, **postmenopausal women** with an LSIL Pap can be managed less aggressively than premenopausal women. Although immediate colposcopy is an option, it is acceptable instead to repeat Pap testing at 6 and 12 months or perform an HPV test and

refer the woman to colposcopy only if the HPV test is positive or any one of the Paps is ASC-US or greater.³⁹

High-Grade Squamous Intraepithelial Lesion

HSIL is an intraepithelial lesion that is encountered in about 0.5% of all Pap samples. Virtually all women (97%)

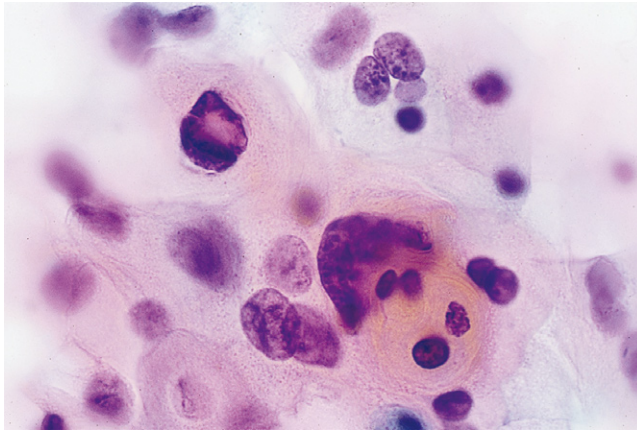


Figure 1.35 Low-grade squamous intraepithelial lesion (LSIL), keratinizing type. A squamous pearl is being formed.

with an HSIL Pap result test positive for high-risk HPV.³⁸ If left untreated, it carries a significant risk of progression to cervical cancer (Table 1.3).

CYTOMORPHOLOGY OF HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION:

- usually parabasal-sized cells
- discrete cells or syncytium-like groups (hyperchromatic crowded groups)
- nuclear atypia
 - enlargement
 - marked irregularity in contour
 - usually marked hyperchromasia
 - marked chromatin coarseness
- keratinizing variant

HSIL is usually a lesion of immature squamous cells. Patten divided HSILs into three categories based on cell size (frequencies in parentheses): large cell (20%), intermediate (70%), and small cell (10%).¹⁵⁷ These subtypes have no biologic significance but are helpful to keep in mind when considering what cells might mimic an HSIL. Nuclear enlargement is generally in the same range as in LSILs, but the nuclear-to-cytoplasmic ratio is higher because the cells are smaller (Fig. 1.37). In general, hyperchromasia, irregular chromatin distribution, and membrane contour irregularity are all more severe than in LSIL. In any given HSIL, one or more of the characteristic nuclear changes may predominate. Thus, some HSILs have irregular nuclear contours but only mild-moderate hyperchromasia. Architecturally, the cells of HSIL are arranged in two main patterns: as distinct individual cells (Fig. 1.37), or as cohesive groups of cells with indistinct cell borders (syncytium-like clusters) (Fig. 1.38). They may have dense, squamoid cytoplasm, but HSIL cells are often completely undifferentiated in appearance and lack any defining squamous features. In fact, cytoplasmic transparency and vacuoles (Fig. 1.39) and an elongated configuration (Fig. 1.40) can cause them to be mistaken for cells of glandular origin. Although usually a lesion of small, immature squamous cells, mature keratinizing cells with marked nuclear atypia are classified as HSIL (Fig. 1.41).

DIFFERENTIAL DIAGNOSIS OF HIGH-GRADE INTRAEPITHELIAL LESION:

- squamous metaplasia
- atrophy
- transitional metaplasia

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Figure 1.36 Management guidelines for women with a Pap showing a low-grade squamous intraepithelial lesion (LSIL). Management may vary if the woman is an adolescent, postmenopausal, or pregnant. (Reprinted with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2008.)

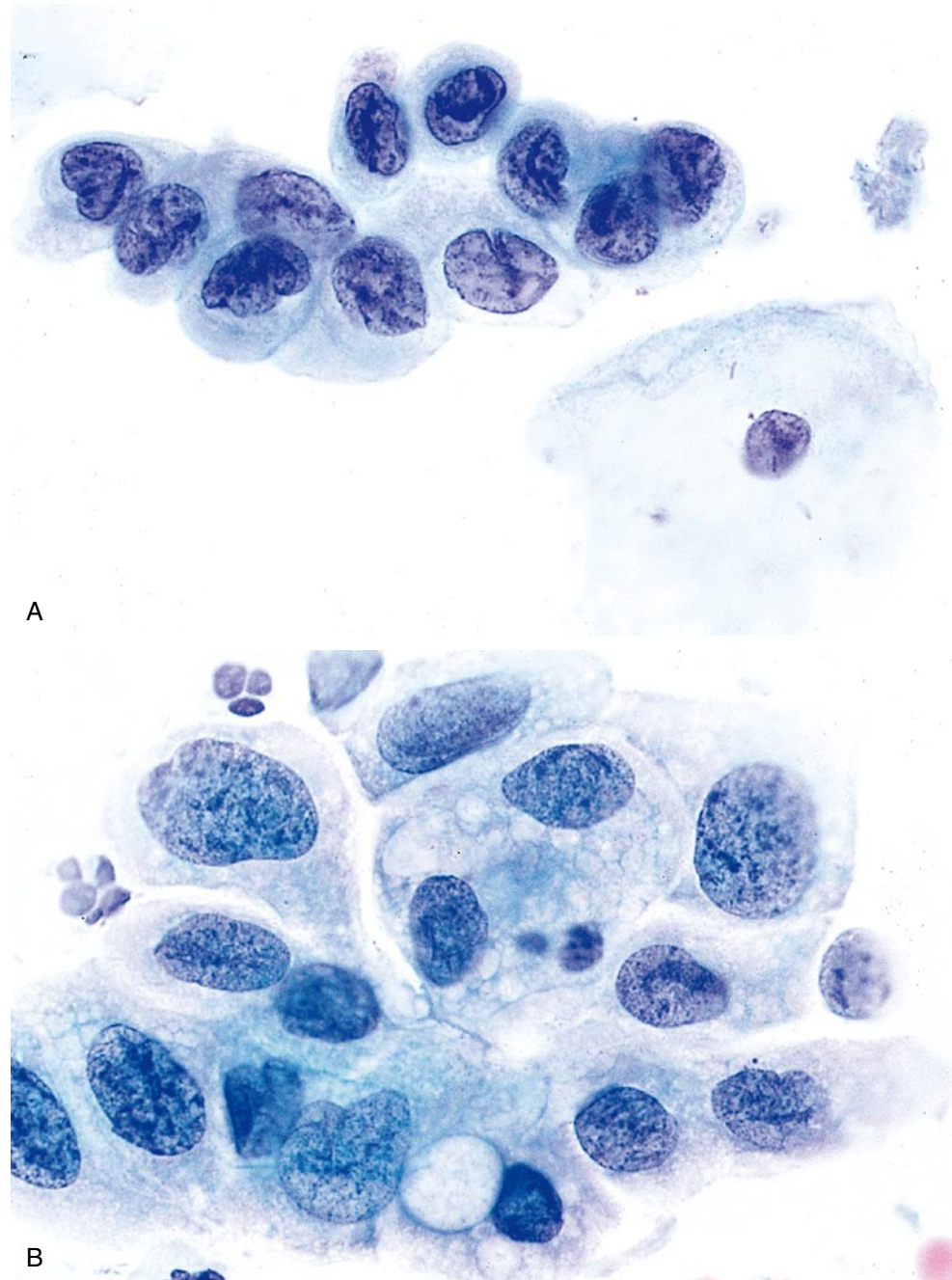


Figure 1.37 High-grade squamous intraepithelial lesion (HSIL). *A*, These cells have scant cytoplasm and a markedly hyperchromatic nucleus with highly irregular nuclear contours. *B*, Cells with a moderate amount of cytoplasm, formerly called “moderate dysplasia” or “CIN 2,” are incorporated in the HSIL category.

- exfoliated endometrial cells
- follicular cervicitis
- histiocytes
- IUD effect
- endocervical polyp atypia
- AIS
- SQC
- atypical squamous cells—cannot exclude HSIL
- ASC-US associated with atrophy (see [Fig. 1.50B-D](#))

Distinguishing HSIL from its many mimics is an important skill of the cytologist. As with histologic sections, one of the most frequent cytologic mimics is squamous metaplasia. Squamous metaplastic cells commonly show only mild nuclear enlargement, nuclear membrane irregularity, and even chromatin coarsening. These changes rarely rise to the level of atypia seen in HSIL. In postmenopausal women, sheets of atrophic squamous epithelium mimic the syncytium-like clusters of HSIL (see [Fig. 1.64](#)). Although atrophic squamous cells have a high nuclear-to-cytoplasmic ratio, their nuclei are usually

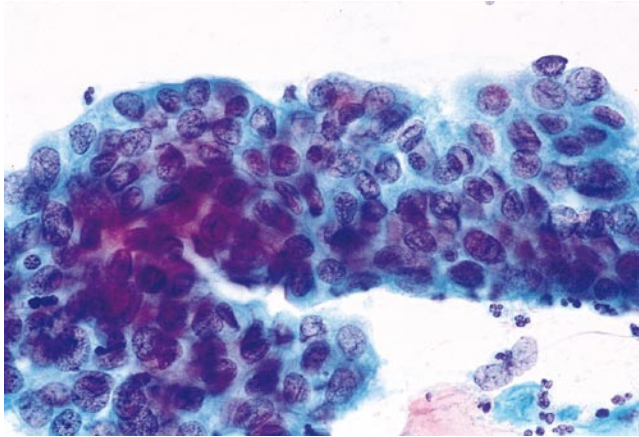


Figure 1.38 High-grade squamous intraepithelial lesion (HSIL). The cells of an HSIL are often arranged in three-dimensional groups in which individual cell borders are indistinct (syncytium-like).

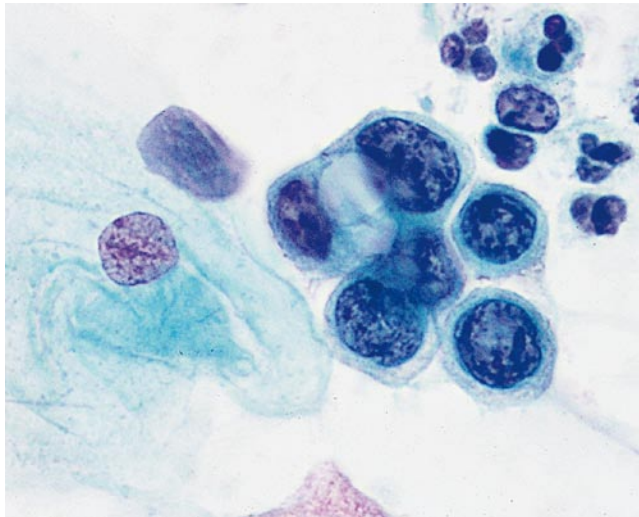


Figure 1.39 High-grade squamous intraepithelial lesion (HSIL). Some HSILs are comprised of small, dispersed, highly atypical cells. The nucleus of these small cells is not much larger than that of normal intermediate cells. They are nevertheless identified as abnormal because of their hyperchromasia, markedly irregular nuclear outline, or both. Some HSIL cells have cytoplasmic vacuoles. These do not indicate a glandular lesion.

regular, with finely textured chromatin. Transitional cell metaplasia, associated with Pap samples from older women, is likely to raise the possibility of HSIL because of the irregularity of the nuclear outlines and prominence of nuclear grooves (see Fig. 1.6B). The absence of hyperchromasia and the abundance of coffee-bean shaped nuclei is a clue to the benign metaplastic nature of these cells. HSIL cells, even those of the small-cell type,¹⁵⁷ are usually bigger than endometrial cells (see Fig. 1.12), vary more in size, and have denser cytoplasm. HSIL clusters are usually less well circumscribed and not as spherical as endometrial cell clusters. Lymphoid cells, commonly seen in postmeno-

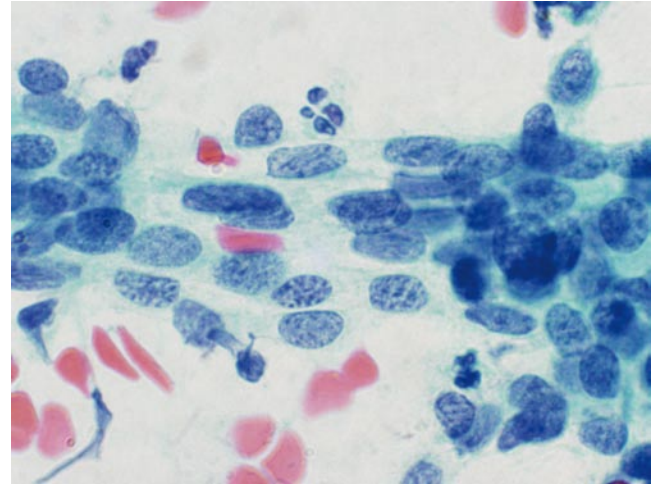


Figure 1.40 High-grade squamous intraepithelial lesion (HSIL). The cells of some HSILs have an elongated configuration that makes them look columnar. In the absence of strips, rosettes, or feathering, this should not be taken for evidence of glandular differentiation (i.e., an adenocarcinoma in situ [AIS]).

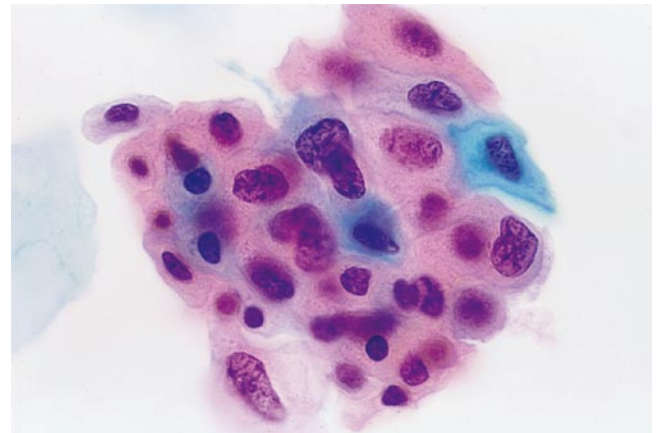


Figure 1.41 High-grade squamous intraepithelial lesion (HSIL), keratinizing type. Although the cells show differentiation by keratinizing, they are classified as HSIL if the nuclei are sufficiently abnormal.

pausal women, are smaller than HSIL cells, their chromatin is even more coarsely textured, and there are often admixed plasma cells, dendritic cells (with a larger, pale nucleus), and tingible-body macrophages (see Fig. 1.16). Histiocytes are roughly the same size as HSIL cells, and many have irregular nuclear contours, but their chromatin is finely textured; they often have abundant fluffy cytoplasm (see Fig. 1.17). The small cells of IUD effect are usually few in number and have a more prominent nucleolus than is commonly seen with HSIL (see Fig. 1.31). Occasional inflamed endocervical polyps are lined by a single layer of highly atypical, hyperchromatic endocervical cells that are easily overinterpreted

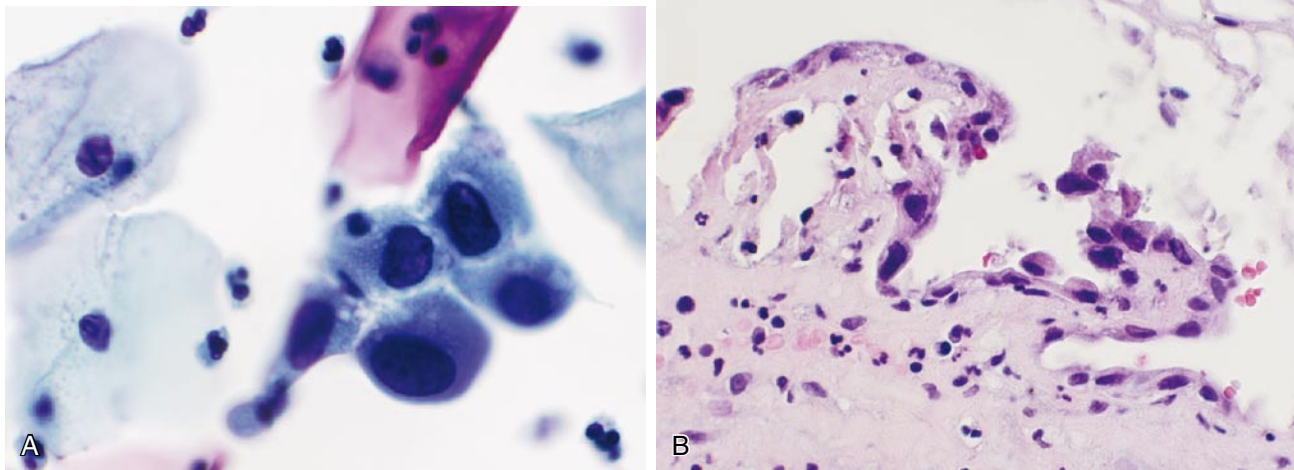


Figure 1.42 Endocervical polyp atypia mimicking HSIL. A, The slide contains scattered isolated cells with dark nuclei. B, The surface of the endocervical polyp reveals a single layer of reactive endocervical cells.

as HSIL. Their true nature is often clarified only after histologic correlation (Fig. 1.42).

The neoplastic cells of AIS share many of the nuclear features of HSIL. Clusters of neoplastic cells are more likely to represent HSIL rather than AIS, unless there is clear columnar differentiation in the form of feathering or rosette formation. SQC should be considered whenever the cytologic criteria for HSIL are fulfilled, but in addition one finds prominent nucleoli or necrotic debris.

In some cases, uncertainty remains regarding the true nature of the cells examined. Cells with the features of squamous metaplasia sometimes show a degree of nuclear atypia that makes it impossible to exclude an HSIL. These “atypical squamous metaplasias” are reported as “ASC, cannot exclude HSIL” (ASC-H). Another diagnostically difficult pattern is the marked squamous atypia associated with a deeply atrophic Pap. Atrophic cervical epithelium sometimes displays a marked squamous atypia that is impossible to distinguish from HSIL. The recommended approach is to call such cases ASC-US.

The recommended management of a woman with an HSIL Pap is illustrated in Figure 1.43. Management is more aggressive than it is for an LSIL Pap, based on the conviction that cytologic HSIL has a higher risk of progression to invasive cancer. With the exception of adolescents and those who are pregnant, an immediate loop electrosurgical excision (the “see-and-treat” approach) is acceptable as the initial treatment if the woman has an HSIL Pap, but not LSIL. An alternative to loop is colposcopy with endocervical assessment (evaluating the canal using the colposcope or tissue sampling). If colposcopy confirms CIN 2,3, the lesion is surgically excised or ablated.¹⁵⁶ If colposcopy is negative (no lesion or only CIN 1), either a diagnostic excisional procedure or observation with colposcopy and Pap testing at 6-month intervals is acceptable, provided that colposcopy is satisfactory and endocervical sampling is negative.³⁹

In **adolescents** with an HSIL Pap, colposcopy is the recommended management. (The “see-and-treat” approach is unacceptable.) If colposcopy confirms CIN 2,3, either treatment or observation for up to 2 years is acceptable, provided that colposcopy was satisfactory.¹⁵⁶ If colposcopy is negative (no lesion or only CIN 1 is confirmed by biopsy), observation with colposcopy and Pap testing at 6-month intervals is recommended, provided that colposcopy is satisfactory and endocervical sampling is negative.³⁹ If HSIL cytology persists for 24 months without histologic confirmation, a diagnostic excisional procedure is recommended. A diagnostic excisional procedure is recommended if colposcopy is unsatisfactory or CIN of any grade is found on endocervical assessment.

In **pregnant women** with an HSIL Pap, it is recommended that colposcopy be performed by a physician experienced with this technique in patients who are pregnant. Biopsy of lesions suspicious for CIN 2,3 or cancer is preferred, and biopsy of other lesions is acceptable. Endocervical curettage is unacceptable. If invasive cancer is suspected, a diagnostic excisional procedure is acceptable. If CIN 2,3 has not been diagnosed histologically, reevaluation with colposcopy and Pap testing is recommended no sooner than 6 weeks postpartum.³⁹

Problems in the Diagnosis of Squamous Intraepithelial Lesions

Avoiding Overdiagnosis of Low-Grade Squamous Intraepithelial Lesions. Care must be taken not to overinterpret nonspecific halos (see Fig. 1.26A and B) or the minimal nuclear changes of benign cells like the PM cells of perimenopausal women (see Fig. 1.25A).¹²⁶ Without hyperchromasia or nuclear membrane irregularity, such cells are best called negative. Cellular changes that include some hyperchromasia or nuclear

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Figure 1.43 Management guidelines for women with a Pap showing a high-grade squamous intraepithelial lesion (HSIL). Immediate loop excision or colposcopy is acceptable for women with an HSIL Pap. The management options may vary if the woman is pregnant, postmenopausal, or adolescent. (Reprinted with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2008.)

membrane irregularity are suggestive of LSIL and should be categorized as ASC-US.

Distinguishing Low-Grade from High-Grade Squamous Intraepithelial Lesions. The distinction between cytologic LSIL and HSIL is an important one, with significantly different implications for clinical management. Proficiency in this distinction is an important skill of the cytology practitioner. As mentioned previously, HSIL is usually a lesion of immature squamous cells, and nuclear atypia (hyperchromasia, irregular chromatin distribution, and membrane contour irregularity) is more severe than in LSIL. If a specimen is composed of both LSIL and HSIL, it should be reported as an HSIL even if the HSIL cells are less numerous than the LSIL cells. In a small percentage of cases, morphologic features intermediate between typical LSIL and HSIL make grading difficult.¹⁵⁸ Although there are generally fewer abnormal cells in an LSIL than in an HSIL, the quantity of cells is an unreliable discriminator.

CYTOMORPHOLOGIC PATTERNS OF “SIL, GRADE CANNOT BE DETERMINED”:

- few dysplastic cells
- extensive cytolysis
- LSIL, with a small number of equivocal HSIL cells
- extensively keratinized SILs, without definite HSIL

Grading is difficult when the dysplastic cells are few in number, when the cytoplasm of the dysplastic cells is obviously affected by cytolysis, or when an LSIL is accompanied by a small number of cells suggestive of

but not conclusive for HSIL. Extensively keratinized SILs without definite HSIL are especially difficult to grade¹⁵⁹ (Fig. 1.44). In all such cases, a diagnosis of “SIL, grade cannot be determined” (or “LSIL, cannot exclude HSIL”) is appropriate.¹⁶⁰ This diagnosis accounts for 3% to 12% of all cytologic SILs.^{155,161–163} Patients with this diagnosis have an intermediate risk (between that of cytologic LSIL and HSIL) of harboring histologic HSIL (CIN 2,3).^{160–162}

Distinguishing High-Grade Squamous Intraepithelial Lesion from Invasive Carcinoma. The criteria used to distinguish HSIL from invasive carcinoma are by no means perfect. Not infrequently, a classic case of HSIL on cytology will turn out to be invasive squamous cancer on biopsy. Conversely, the possibility of invasive cancer is often raised in cases of HSIL in which the cells have marked nuclear abnormalities associated with abundant, heavily keratinized cytoplasm and unusual cell shapes, but the lesion turns out to be only a keratinizing HSIL on biopsy.⁷⁸ Physicians understand that no diagnosis of HSIL on cytologic material excludes the possibility of invasive cancer, and that colposcopy and biopsy are necessary for confirmation. Some HSILs with features worrisome for invasive cancer can be reported as “HSIL, with features suggestive of invasive cancer.”

Squamous Cell Carcinoma

SQC is the most common malignant tumor of the cervix, accounting for about 75% of cervical cancers.¹⁶⁴ Although most patients are between the ages of 35 and

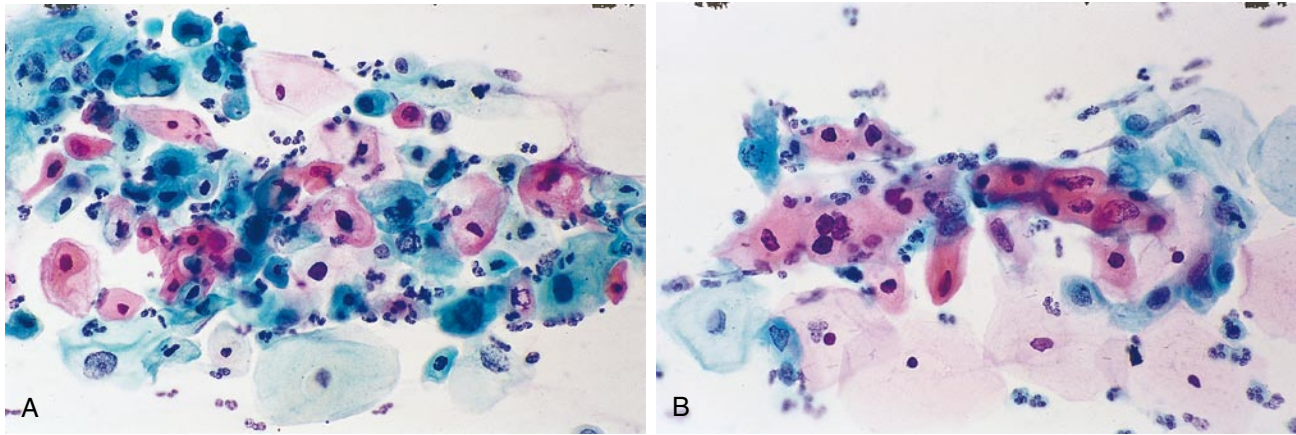


Figure 1.44 Squamous intraepithelial lesion (SIL), cannot determine grade. When a lesion is extensively keratinized and there is no definite high-grade squamous intraepithelial lesion (HSIL), it is difficult to grade. Colposcopically directed biopsies showed A, CIN 1 and B, CIN 2,3.

55 years, invasive tumors occur in younger patients, including those under 30 years of age.⁶⁷ HPV 16 accounts for about 50% to 60% of SQCs worldwide, and HPV 18 for an additional 10% to 15%.¹⁶⁵ Early invasive tumors can be asymptomatic, but as the tumor grows patients may develop abnormal vaginal bleeding, vaginal discharge, and pain during intercourse (dyspareunia). With more advanced tumors there can be back pain, sciatica, tenesmus, and hematuria.

Histologically and cytologically, SQCs range from well-differentiated, keratinizing tumors to poorly differentiated, nonkeratinizing tumors.¹⁶⁶ Some SQCs cannot be distinguished cytologically from HSIL, particularly the smaller, less deeply invasive tumors.¹⁶⁷ Others can be confidently diagnosed as invasive cancers, however.

CYTOMORPHOLOGY OF SQUAMOUS CELL CARCINOMA:

- HSIL features, plus:
 - macronucleolus
 - irregular chromatin distribution
 - tumor diathesis
- “tadpoles” and “fiber cells” (keratinizing type)

The classic pattern of SQC shows abundant necrotic debris: a granular, amorphous precipitate with nuclear debris and red blood cells called “tumor diathesis” (Fig. 1.45). It is not specific for invasive cancers; a similar pattern is seen in some atrophic smears and even during heavy

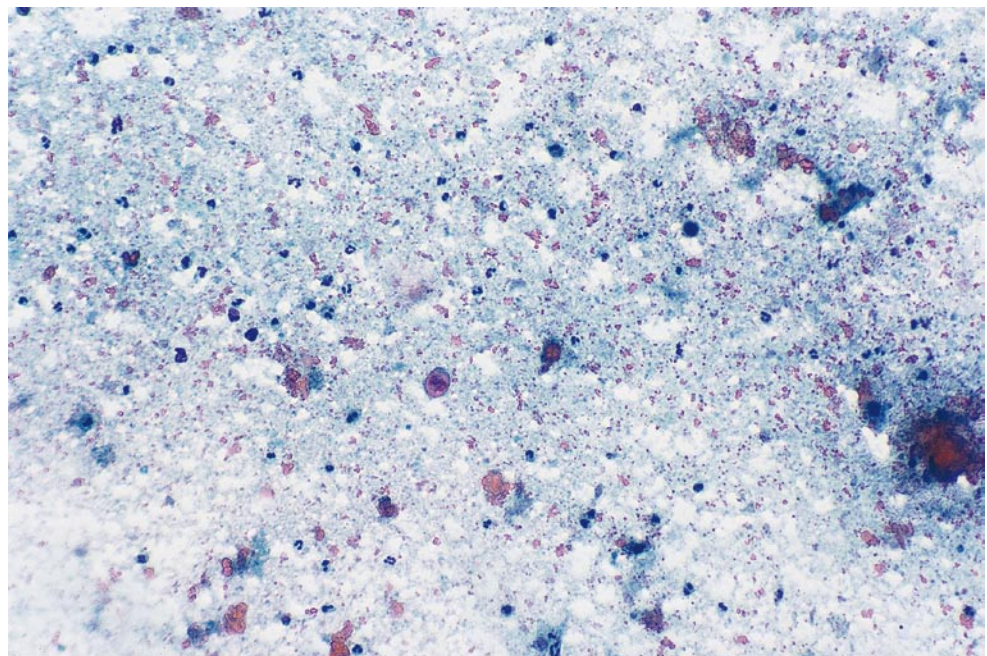


Figure 1.45 Squamous cell carcinoma (SQC). Slides from deeply invasive tumors show abundant tumor diathesis, a granular precipitate of lysed blood and cell fragments. In such cases, the malignant cells can be hard to identify. In other cases, the tumor diathesis is focal, and, if missed, the case is misclassified as a high-grade squamous intraepithelial lesion (HSIL).

menstrual bleeding. When associated with hyperchromatic crowded groups of atypical cells or abundant atypical keratinized cells with unusual shapes (“tadpoles,” “fiber cells”), the pattern is diagnostic.

The cells of a nonkeratinizing SQC look like modified HSIL cells (Figs. 1.46, 1.47). Like HSIL, they are hyperchromatic and have scant cytoplasm, but they have a prominent nucleolus and a highly irregular pattern of chromatin distribution. The cells of a keratinizing SQC are often bizarrely elongated (Fig. 1.48). Some are long and spindle shaped, with small condensed nuclei (“fiber cells”). Others have a larger cytoplasmic body with a long tail (“tadpole cells”). Such cells are uncommon in keratinizing HSILs.

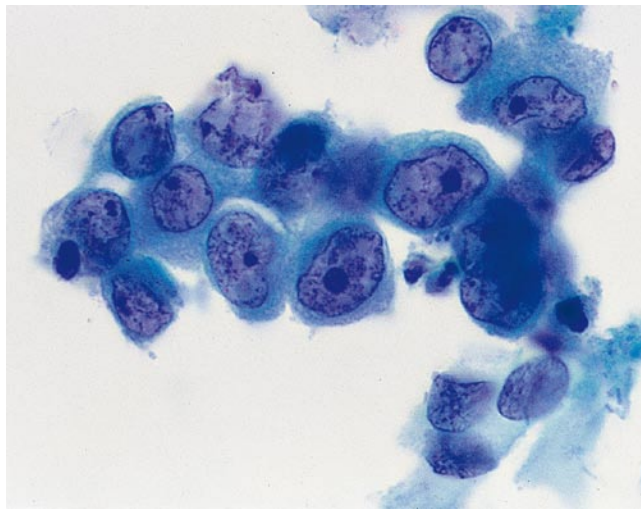


Figure 1.46 Squamous cell carcinoma (SQC), nonkeratinizing. The malignant cells have irregularly distributed chromatin and a prominent nucleolus, characteristic features of invasive SQCs.

Most SQCs are associated with an adjacent or overlying HSIL, and therefore cytologic preparations from SQCs often contain a population of HSIL cells as well.

DIFFERENTIAL DIAGNOSIS OF SQUAMOUS CELL CARCINOMA:

- HSIL
- atypia of atrophy
- atypia of repair
- benign endometrial cells
- Behçet disease
- pemphigus vulgaris

The differential diagnosis of SQC includes HSIL. Prominent nucleoli and tumor diathesis are the principal cytologic features that help distinguish SQC from HSIL, but these features are not present in all smears from patients with SQC. A significant number of women with SQC are diagnosed as having HSIL because prominent nucleoli and tumor diathesis are absent.¹⁶⁷ Conversely, a granular, tumor diathesis-like background is not specific for invasive cancers and is seen in women with atrophic vaginitis⁷⁷ (see Fig. 1.7), severe cervicitis, and rare cases of HSIL.⁷⁸

In postmenopausal women, marked atrophy atypia is one of the most common benign mimics of a keratinizing SQC (see Fig. 1.50B-D). The benign atypia of atrophy contains scattered cells with large, dark nuclei and eosinophilic or orangeophilic cytoplasm. Their large, dark nuclei are alarming, but chromatin is usually smudgy. Such cells, if seen in a deeply atrophic squamous background, should be interpreted as ASC-US and not HSIL or invasive cancer.

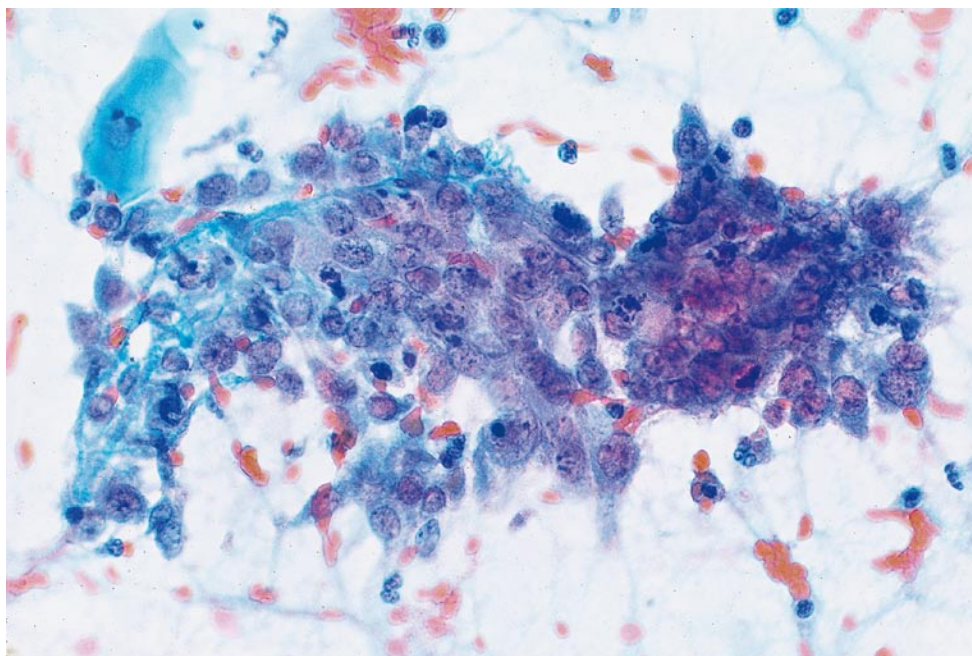


Figure 1.47 Squamous cell carcinoma (SQC), nonkeratinizing. The sheetlike arrangement of poorly differentiated squamous carcinoma cells with nucleoli and mitoses mimics the appearance of reparative epithelium, but the crowding and haphazard arrangement of the cells are not typical of repair.

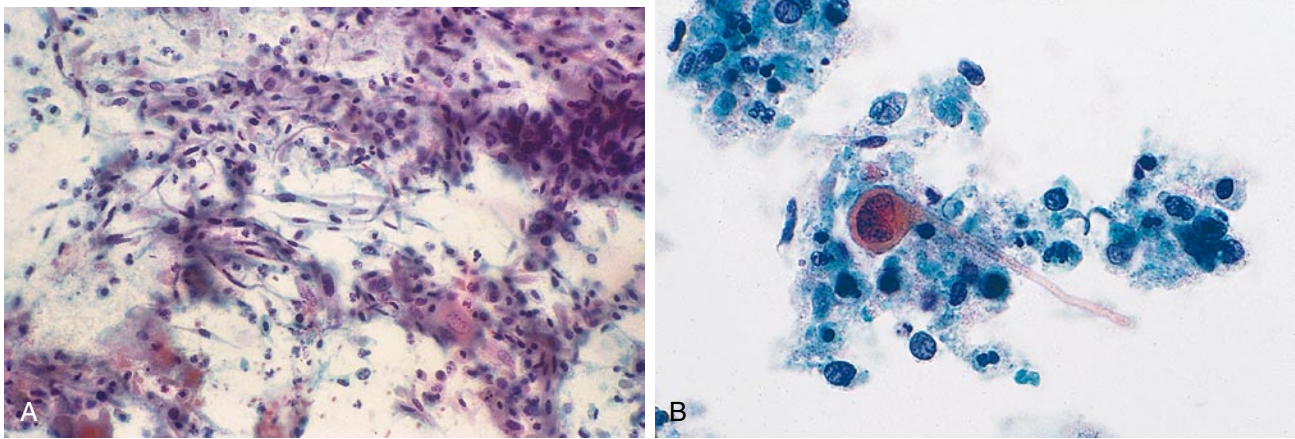


Figure 1.48 Squamous cell carcinoma, keratinizing. A, In keratinizing carcinomas, the cells have markedly aberrant shapes, as seen here. "Fiber cells" are numerous. B, A tadpole cell and some tumor diatheses are seen in this tumor.

Marked repair atypia is another good mimic of nonkeratinizing SQC (see Fig. 1.52). Both repair and SQC contain large cells with prominent nucleoli, and mitoses are seen in both. Repair cells are recognized by their finely textured chromatin pattern, the flatness and cohesion of the sheets. If the nuclei have coarsely textured chromatin, show marked crowding, or demonstrate significant dyshesion, SQC should be considered.

A minority of nonkeratinizing SQCs are composed of small cells that are indistinguishable from endometrial cells (see Fig. 1.13B). The blood that accompanies menstrual endometrial cells resembles the granular necrosis that is tumor diathesis, adding to the similarity. Mitoses, if identified, should raise the suspicion of SQC. In some cases, knowledge that the patient has a suspicious cervical mass or suspicious clinical symptoms (e.g., dyspareunia) may be the only clue to the correct interpretation.

Behçet disease, a chronic disease of uncertain cause that is characterized by oral and genital ulcers, can mimic SQC. Smears may show numerous isolated, keratinized cells with dark, pleomorphic nuclei and large nucleoli. A history of this disorder may be critical for correct diagnosis.¹⁶⁸ Smears from patients with pemphigus vulgaris, a blistering disorder that involves mucosal surfaces, may mimic a poorly differentiated SQC. A complete history may be important to avoid making an overcall, although cases of coexisting SQC and pemphigus vulgaris have been reported.¹⁶⁹

Treatment choices for women with cervical cancer include surgery (hysterectomy plus lymphadenectomy), radiation therapy, and chemoradiation, depending on tumor stage.¹⁷⁰ Hysterectomy (simple or radical, depending on histologic findings) is the treatment of choice for early-stage (IA1, IA2, and IB1), nonbulky disease. Women with early-stage disease who wish to preserve fertility have the option of trachelectomy

(surgical removal of the cervix) with lymph node dissection. If intermediate- or high-risk histologic features are found after hysterectomy, postoperative radiotherapy (with or without chemoradiation) improves local control and survival. Patients with higher stage disease (IB2 to IVA) are likely to be treated with external beam and intracavitary radiation combined with cisplatin-based chemotherapy.¹⁷⁰ Women with metastatic cancer (stage IVB) are best treated with systemic chemotherapy, with radiation therapy reserved for palliation of symptomatic pelvic disease.

Atypical Squamous Cells

Since the days of Papanicolaou, cytology laboratories have used a borderline category to report findings of uncertain significance. Terminology was inconsistent and often confusing, however, because benign changes were sometimes reported as "benign atypia." In the Bethesda System, recognizably benign cases, previously called "benign atypia," "inflammatory atypia," or "reactive atypia," are excluded from this category. The 1988 and 1991 Bethesda Systems used the term *atypical squamous cells of undetermined significance* to designate "cellular abnormalities that were more marked than those attributable to reactive changes but that quantitatively or qualitatively fell short of a definitive diagnosis of SIL." In the 2001 Bethesda System, ASC-US was replaced by ASC and redefined in a subtle way. Instead of being a diagnosis of exclusion, ASC is a diagnosis conveying a suspicion of SIL.

Most cytologists agree that this category is essential. Eliminating ASC would result in increased reporting of LSIL (which probably contributes little to cancer prevention) and decreased reporting of HSIL.¹⁷¹ It is risky to eliminate an equivocal category because of the large number of women with underlying HSIL who are

discovered through a workup for an equivocal cytology reading. In fact, histologic HSIL is found in 10% to 20% of women with ASC Paps,^{38,155} and, ironically, ASC Pap samples, because they are more common, detect more cases of HSIL than HSIL Paps.¹⁷² Finally, the elimination of an equivocal category seems imprudent given the expectations in the United States for a sensitive Pap test.⁸⁵

ASC diagnoses should be kept to a minimum. There is no correct rate of ASC, but expert consensus suggests that it be kept to less than 5% of all Pap cases. For labs that serve high-risk populations, a better gauge is the ASC/SIL ratio, which should not exceed 3:1.⁸⁸ The ASC rate can be kept low through education, optimal sample preparation, and the monitoring (with feedback) of individual ASC/SIL ratios.^{173,174} According to a 2003 College of American Pathologists survey, most labs are complying with the above recommendations: ASC accounts for about 4% of all Pap samples, and the median ASC/SIL ratio in the United States is 1.4.⁸⁷

The 2001 Bethesda System differs from the 1991 Bethesda System in the way ASC cases are subclassified. In the 1991 Bethesda System, ASC (formerly ASC-US) was subclassified as “favor reactive,” “favor SIL,” or “not otherwise specified.” In many laboratories, this provided useful risk stratification.^{175–177} In the 2001 Bethesda System, however, the favor reactive qualifier was eliminated. Because of an increased focus on the detection of high-grade disease (and a relative emphasis away from the detection of LSIL, perceived as a self-limited infection by HPV), it was proposed that the newly renamed ASC cases be subqualified as either “**of undetermined significance**” (ASC-US) or “**cannot exclude HSIL**” (ASC-H). The latter category had already been in use in some laboratories, and the higher risk associated with it was well recognized.^{176,178}

Atypical Squamous Cells of Undetermined Significance

The cases described in this section are daily dilemmas for cytologists. The decision to categorize a Pap as negative (NILM), ASC-US, ASC-H, LSIL, or HSIL rests on the quantity of the altered squamous cells, the severity of the abnormalities, the state of preservation of the specimen, and the clinical setting. If the changes are suspicious but not conclusive for SIL, the findings are reported as ASC-US.

CYTOMORPHOLOGIC PATTERNS OF ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE:

- atypical cells with “mature” intermediate-type cytoplasm, including cells suggestive of koilocytes
- ASC in atrophy
- atypical parakeratosis
- atypical repair
- “atypia” resulting from a compromised specimen

Atypical “mature” squamous cells with features suspicious for a SIL are classified as ASC-US (Fig. 1.49A). Some cases have some but not all of the features of HPV effect, such as binucleation with minimal hyperchromasia (Fig. 1.49B).

ASC associated with atrophy are diagnosed as ASC-US when there is nuclear enlargement with hyperchromasia, when nuclei are irregular in contour and chromatin distribution, and when there is marked cellular pleomorphism with unusual shapes. In extreme cases, the changes seen in atrophy with inflammation are difficult to distinguish from a SIL or invasive cancer (Fig. 1.50). The management options include a course of intra-vaginal estrogen cream (e.g., 1 g estrogen cream three

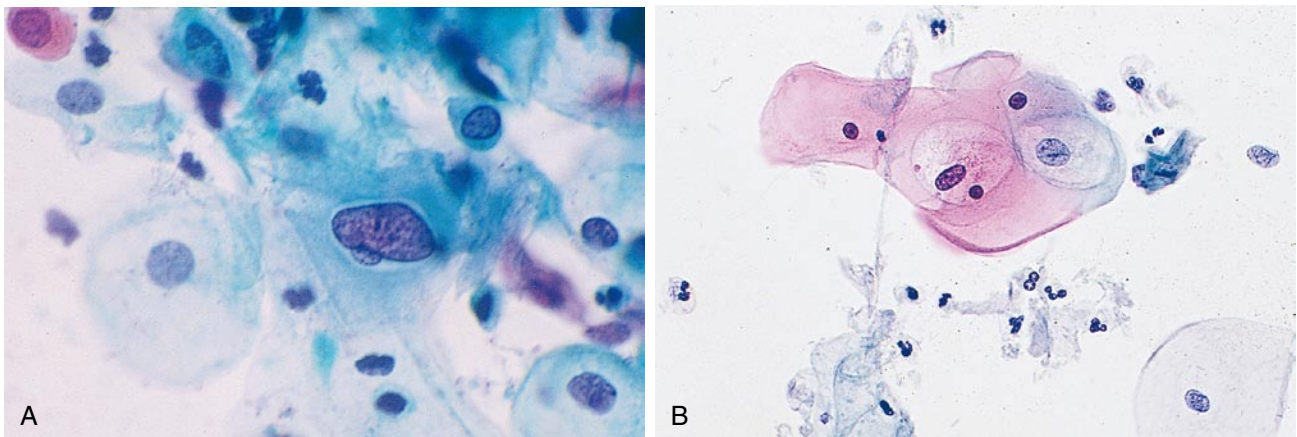


Figure 1.49 Atypical squamous cells of undetermined significance (ASC-US). A, The nucleus of this mature squamous cell is significantly enlarged and there is moderate hyperchromasia. Cells like this, particularly if few in number, are suggestive but not diagnostic of a squamous intraepithelial lesion (SIL). B, Some cells have large cytoplasmic cavities but minimal nuclear atypia. It is preferable to diagnose such cases as ASC-US when abnormal cells are few and the changes minimal.

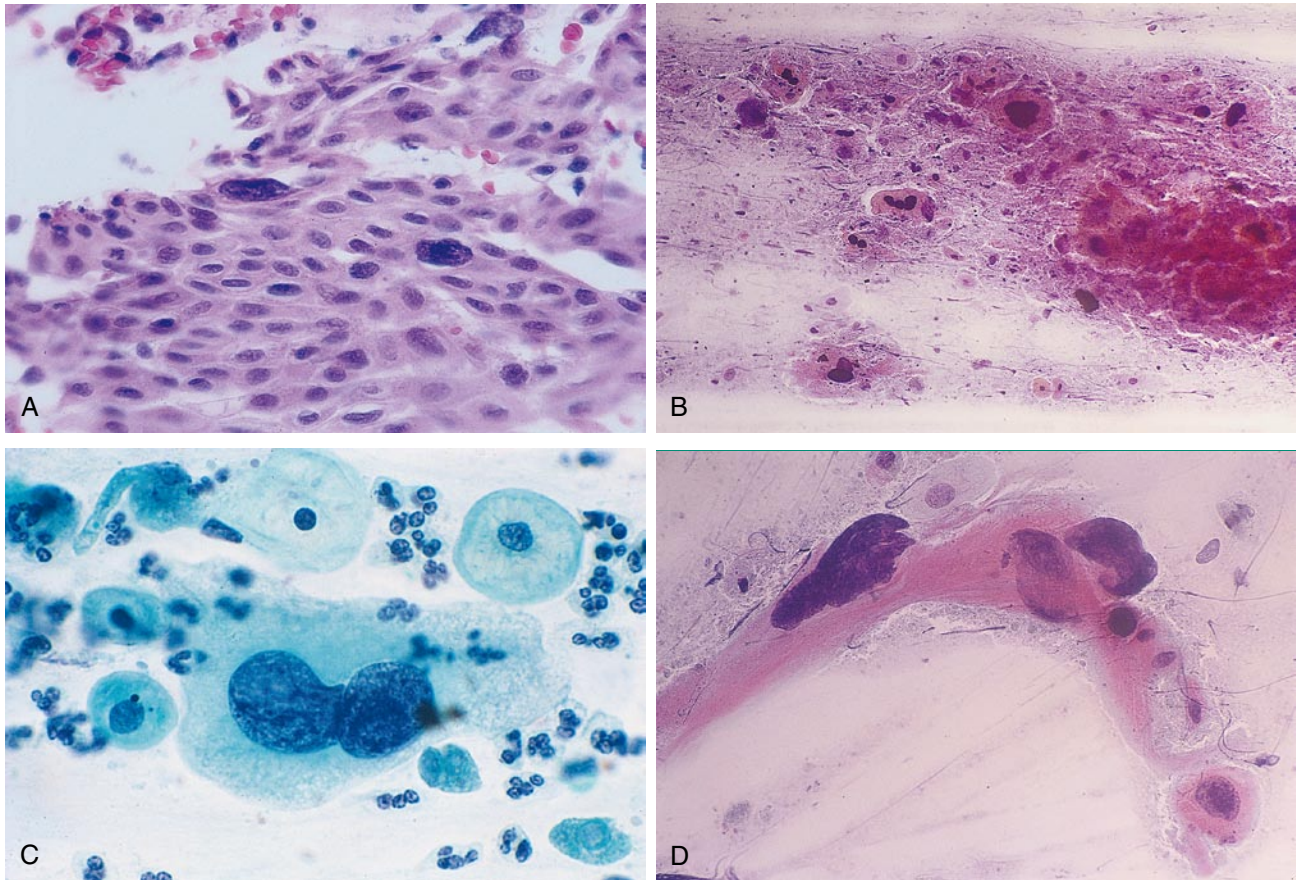


Figure 1.50 Atypical squamous cells of undetermined significance (ASC-US), associated with atrophy. A, Histologic section of benign atrophy-associated atypia. B, Cytologic smear shows scattered large atypical cells in a granular background. C, Some cells have a markedly enlarged, hyperchromatic nucleus. D, Often cells are poorly preserved, with smudgy nuclei and hypereosinophilic cytoplasm. Follow-up in all cases was benign.

times a week for several months), followed by a repeat Pap test a week after completing the regimen.¹⁷⁹ A significant squamous lesion will be more easily detected among the mature cells, whereas a benign “atypia” resulting from atrophy will be transformed into normal epithelium.

Squamous atypia in a postmenopausal woman is less often associated with a biopsy-proven SIL (17%) than in a premenopausal woman (46%).¹⁸⁰ Further, the rate of HPV detection in women with atypia is lower (10% versus 50%). In another study, squamous atypia in women over the age of 50 was associated with histologic SIL in less than 5% of cases.¹⁸¹

Parakeratosis with mild nuclear enlargement and mild to moderate nuclear membrane irregularity (atypical parakeratosis) suggests an SIL (Fig. 1.51). In some cases such cells are accompanied by other changes diagnostic of an SIL, but when the changes are mild, such cases are best classified as ASC-US.

Highly exuberant atypical repair reactions can demonstrate cellular crowding and overlap (in contrast with typical repair, which is in flat sheets), marked variation in nuclear size, prominent and irregular nucleoli, and irregular chromatin distribution (Fig. 1.52). Such cases

are difficult to distinguish from invasive carcinoma. Carcinomas often have a tumor diathesis and many isolated atypical cells, features that are usually absent in repair reactions.

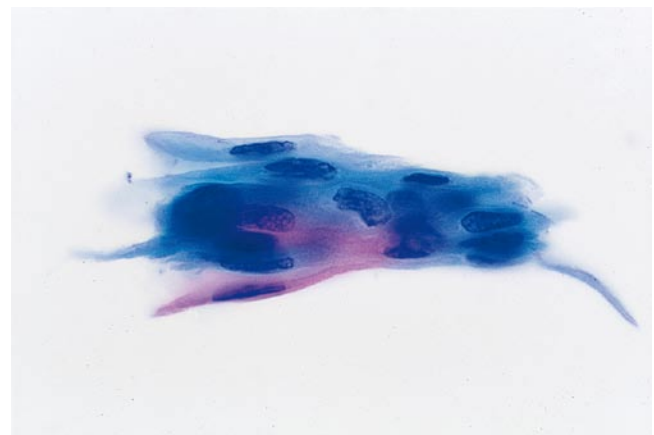


Figure 1.51 Atypical squamous cells of undetermined significance (ASC-US), with features of atypical parakeratosis. Small, keratinized squamous cells with mild variation in nuclear size and contour may represent either a reactive process or a significant squamous lesion.

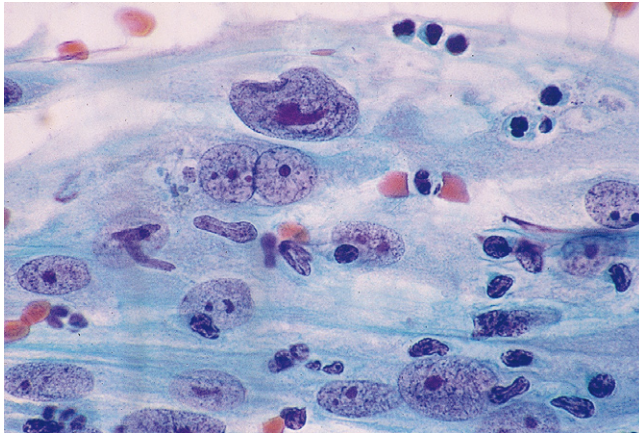


Figure 1.52 Atypical squamous cells of undetermined significance (ASC-US), atypical repair reaction. In some cases of repair there is such striking nuclear atypia that an invasive cancer cannot be excluded. This lesion proved to be benign.

The recommended management of women with an ASC-US Pap is illustrated in [Figure 1.53](#). When liquid-based cytology is used, or when co-collection can be carried out with conventional smears, so-called “reflex” HPV DNA testing is the preferred approach. Women who test positive should be referred for colposcopy with directed biopsies. Women who test negative return to a schedule of regular Pap testing. Reflex HPV testing is the preferred approach because it is more sensitive than a single repeat Pap test.³⁸ If repeat Pap testing is selected, a repeat Pap should be performed every 6 months until two consecutive negative results are obtained, at which point the patient can return to routine annual testing. If any of the subsequent Paps shows ASC-US or worse, the patient should be referred

for colposcopy. If immediate colposcopy is selected and colposcopic examination is negative, she can return to a schedule of annual Pap testing.

There are minor variations in the recommendations for adolescents and pregnant women. In adolescents with an ASC-US Pap, follow-up with annual Pap testing is recommended, and only women with an HSIL Pap (or worse) should be referred to colposcopy. The recommendations for pregnant women are the same as the general recommendations, except that it is acceptable to defer colposcopy until 6 weeks postpartum.

Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion

ASC-H is the other, less common subtype of ASC, representing 5% to 10% of all ASC cases.⁸⁷ This category is reserved for Pap samples that are specifically *suspicious for HSIL*. The most common pattern is that of immature (small) squamous cells with mild to moderate nuclear atypia (enlargement, hyperchromasia, membrane irregularity), commonly called atypical squamous metaplasia ([Figs. 1.54, 1.55](#)).

ASC-H has a positive predictive value for histologic CIN 2,3 that is significantly higher than that of ASC-US (50% vs 17%).¹⁸² For this reason, women with an ASC-H Pap should be referred for colposcopy ([Fig. 1.56](#)). If histologic CIN 2,3 is not identified, follow-up with either repeat Pap at 6 and 12 months or HPV DNA testing at 12 months is acceptable. If she has ASC-US or worse on her repeat Pap or tests positive for high-risk HPV, the patient should be referred for another colposcopic examination.³⁹

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Figure 1.53 Management guidelines for women with atypical squamous cells of undetermined significance (ASC-US). Human papillomavirus (HPV) testing is preferred if liquid-based cytology or co-collection is available (“reflex HPV testing”). (Reprinted with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2008.)

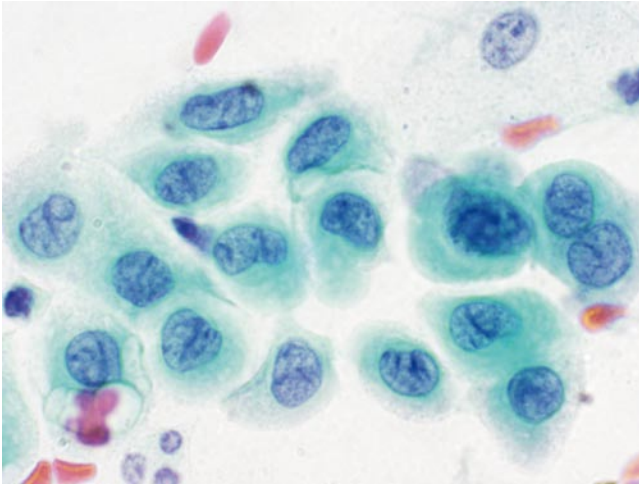


Figure 1.54 Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H). These metaplastic-like cells show significant nuclear membrane irregularity. There is no hyperchromasia or significant nuclear size variation, however, which makes the diagnosis of high-grade squamous intraepithelial lesion (HSIL) uncertain.

GLANDULAR ABNORMALITIES

Endocervical Adenocarcinoma in Situ

Endocervical AIS is the recognized precursor to endocervical adenocarcinoma. The evidence linking them is similar to that linking SIL to SQC: Women with AIS are an average 13 years younger than those with adenocarcinoma (39 versus 52 years old); AIS resembles adenocarcinoma morphologically and is often found in histologic sections adjacent to invasive carcinoma; AIS has been discovered retrospectively in biopsies originally called negative in women who later develop

invasive adenocarcinomas;¹⁸³ and HPV 16 and 18 have been identified in AIS and adenocarcinomas in similar proportions.

The concept of endocervical AIS was first introduced in 1953 when its histologic features were convincingly illustrated by Friedell and McKay.¹⁸⁴ Widespread recognition of the cytologic characteristics of AIS came only in the late 1970s and 1980s, when a group of Australian investigators published their experience with a large number of histologically confirmed cases.^{185–187} It was not until 2001 that the cytologic criteria for AIS were considered sufficiently reliable to merit a separate, explicit diagnostic category in the Bethesda System. There has been a steady increase in the incidence of AIS between 1975 and 1995, as a result in part of better cytologic recognition of AIS. But cytologic diagnosis remains a challenge, mainly because it is still an uncommon lesion. The incidence of AIS is a mere 0.61/100,000, which is 2% that of CIN 3. Therefore, in practice, one is likely to see one case of AIS for every 50 cases of HSIL.

CYTOMORPHOLOGY OF ADENOCARCINOMA IN SITU:

- hyperchromatic crowded groups
- glandular differentiation
 - columnar cells
 - strips and rosettes
 - “feathering”
- neoplastic nucleus:
 - hyperchromasia
 - crowding, stratification
 - inconspicuous nucleolus
 - apoptosis
 - mitoses
 - no tumor diathesis

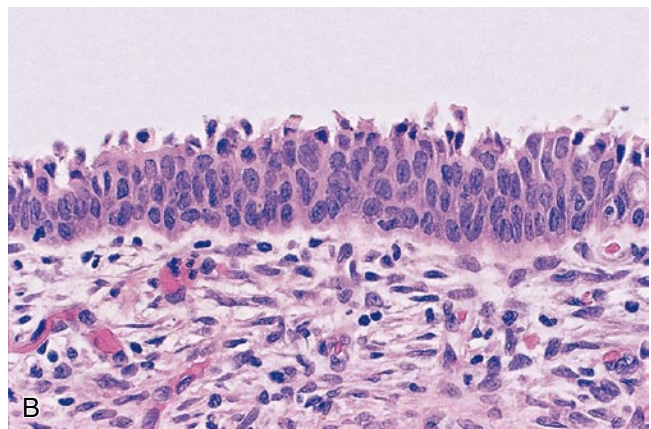
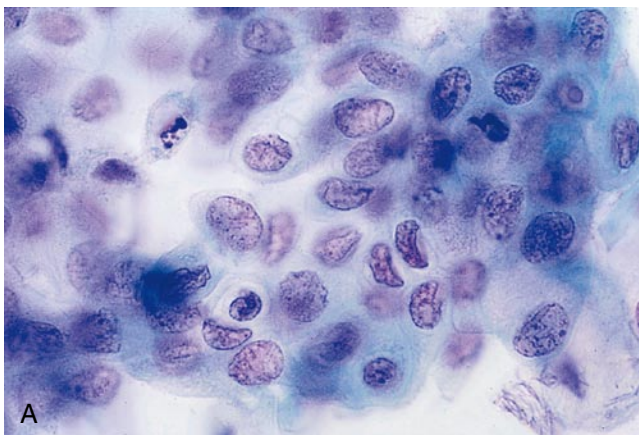


Figure 1.55 Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H). A, Immature squamous metaplastic cells sometimes show some nuclear atypia that raises the possibility of high-grade squamous intraepithelial lesion (HSIL), but the degree of nuclear enlargement, hyperchromasia, and membrane irregularity is insufficient for a definite diagnosis. B, Subsequent colposcopy revealed benign immature squamous metaplasia, and a human papillomavirus (HPV) test on the residual ThinPrep vial was negative for high-risk HPV.

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Figure 1.56 Management guidelines for women with atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H). ASC-H warrants immediate colposcopy. (Reprinted with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2008.)

In the 2001 Bethesda System, AIS is a separate diagnostic category because there is a consensus that the cytologic criteria are accurate and reproducible.^{187–189} Examination of the slide under low magnification reveals hyperchromatic crowded groups similar to those of HSIL (Fig. 1.57). Closer inspection reveals evidence of glandular differentiation: columnar cells arranged in strips or rosettes (Fig. 1.58A). Columnar

cells in sheets reveal their glandular nature by “feathering,” a splaying out around the edges (Fig. 1.58B). Nuclei are hyperchromatic and crowded and there is scant cytoplasm. Apoptotic bodies are seen in most cases and are a useful clue to the diagnosis.¹⁹⁰ Mitoses are seen in some cases and are helpful, but only if accompanied by the typical nuclear changes previously described.

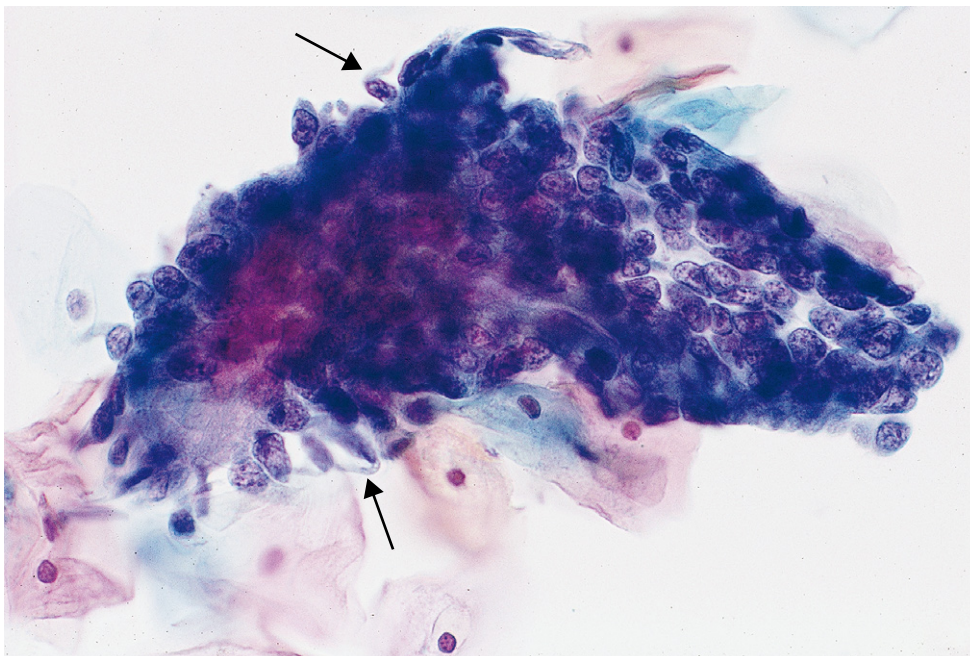


Figure 1.57 Adenocarcinoma in situ (AIS). At first glance, some groups of neoplastic cells resemble the hyperchromatic crowded groups of a high-grade squamous intraepithelial lesion. Only slight feathering is seen (arrows).

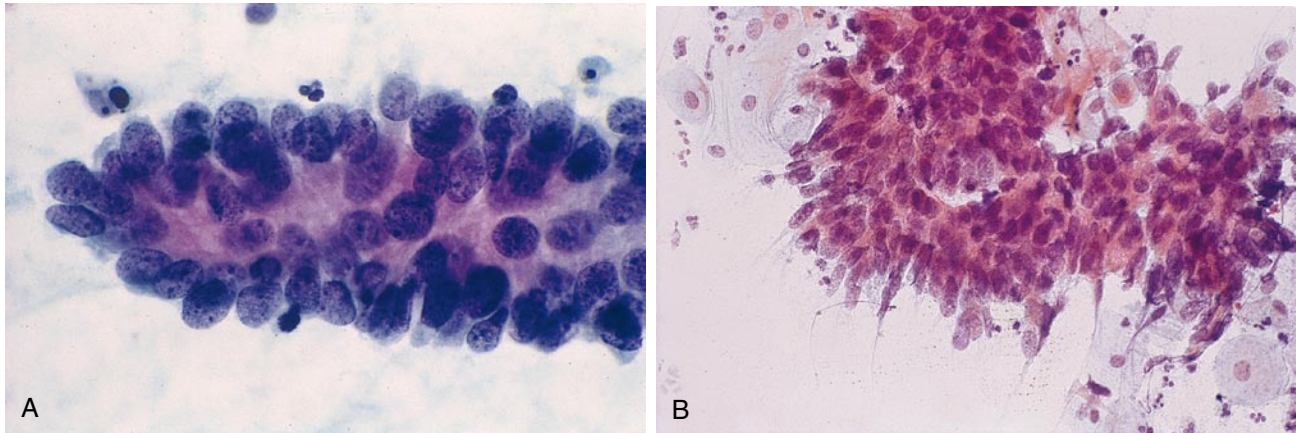


Figure 1.58 Adenocarcinoma in situ (AIS). A, Rosettes are highly characteristic of AIS and virtually never seen with high-grade squamous intraepithelial lesion (HSIL), benign endocervical cells, or lower uterine segment (LUS) or endometrial epithelium. B, The glandular nature of these neoplastic cells is betrayed by “feathering.”

DIFFERENTIAL DIAGNOSIS OF ADENOCARCINOMA IN SITU:

- exfoliated endometrial cells
- tubal metaplasia
- abraded endometrial cells and LUS
- reactive endocervical cells
- reparative changes
- HSIL
- invasive adenocarcinoma

A serious, and not uncommon, problem is mistaking AIS for benign cells.^{191,192} Some cases of AIS, in fact, strongly resemble menstrual endometrial cells (“endometrioid” AIS),¹⁹³ and apoptosis is a feature of both (see Fig. 1.13C). The cells of AIS are generally better preserved and have a coarser chromatic texture. Feathering, rosettes, and mitoses are virtually never seen in menstrual endometrium. AIS resembles tubal metaplasia (Fig. 1.59), but tubal metaplasia is recognized by the presence of terminal bars and cilia. In addition, tubal metaplasia lacks mitoses and apoptosis. AIS also strongly resembles directly sampled endometrium or LUS, particularly because mitoses can be seen in both (see Fig. 1.14B and C). The intact tubules with stromal fragments typical of endometrium or LUS epithelium are rarely seen in AIS (Fig. 1.14A), however, and the nuclei of endometrium or LUS, although crowded, are arranged in an orderly, rather than haphazard pattern.

Reactive endocervical cells and reparative epithelia show a greater range of nuclear size and less hyperchromasia than AIS, which generally has strikingly uniform dark nuclei; the nuclei of reactive endocervical cells typically have prominent nucleoli, a feature seen in only a small proportion of AIS cases.

AIS can resemble HSIL almost to perfection (see Fig. 1.57). Both are characterized by hyperchromatic crowded groups, mitoses, apoptosis, and coarse chromatin. The cells of HSIL, like those of AIS, can have pale or foamy cytoplasm. The diagnosis of AIS should be reserved for

cases with clear columnar glandular differentiation: strips of columnar cells, rosettes, and feathering.

The distinction between AIS and adenocarcinoma is problematic. Some cases of adenocarcinoma are clearly invasive because the cells are large, with abundant cytoplasm and prominent nucleoli, and a tumor diathesis is present. These features are absent in some cases of adenocarcinoma, however, resulting in significant morphologic overlap between AIS and cervical adenocarcinoma. Thus, the cytologic diagnosis of AIS does not exclude invasive adenocarcinoma; histologic evaluation is necessary for a definite distinction. Even histologic distinction between AIS and adenocarcinoma is often a difficult judgment based in part on whether the lesion extends below the normal location of endocervical glands.

Consensus guidelines recommend that a woman with a cytologic diagnosis of AIS undergo colposcopic examination with endocervical sampling. For women older than 35 years and younger women with unexplained vaginal bleeding, endometrial sampling should be included. If there is no evidence of invasive disease, she should have a diagnostic excision procedure, one that yields an intact specimen with interpretable margins.³⁹

Adenocarcinoma

Adenocarcinomas of the endocervix, endometrium, vagina, and even the ovaries and fallopian tubes are sometimes detected with the Pap test. There is significant overlap in their morphologic features, so that a precise site of origin often cannot be established. Additional testing (imaging studies, histologic sampling) is usually required for definitive classification and treatment.

Endocervical Adenocarcinoma

Adenocarcinoma of the endocervix represents approximately 15% of cervical cancers in the United States.¹⁶⁴ Some patients complain of bleeding or vaginal discharge, but others are asymptomatic. As with cervical SQCs, HPV

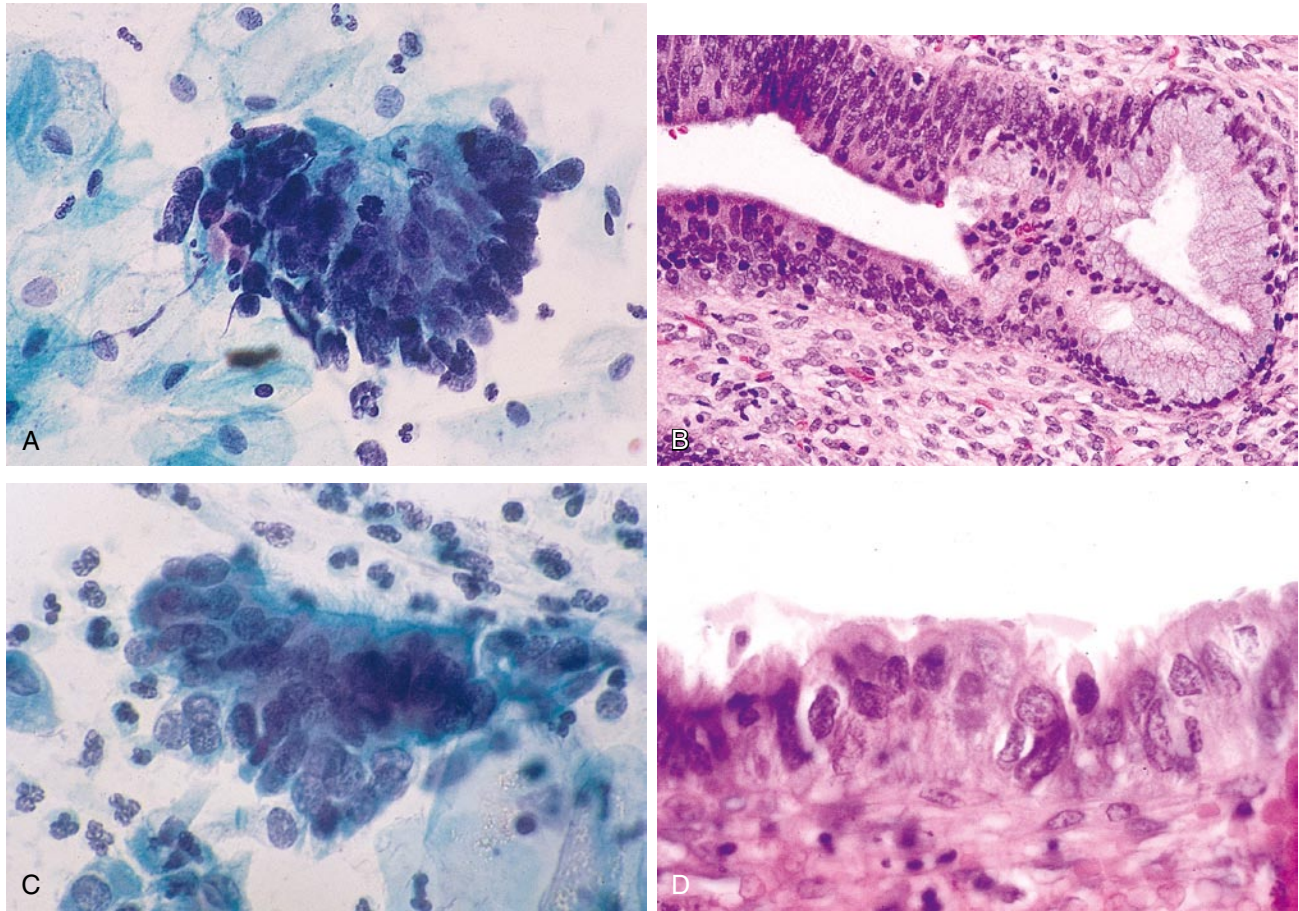


Figure 1.59 Adenocarcinoma in situ (AIS) compared to tubal metaplasia. A, Endocervical AIS. Cells are columnar in shape, dark, crowded, and arranged in a curved strip. B, A cone biopsy revealed AIS. C, Tubal metaplasia. Atypical glandular cells bear a resemblance to those in A, except that cilia are identified. D, Subsequent biopsies showed tubal metaplasia of surface endocervical epithelium.

is commonly present in endocervical adenocarcinomas. HPV 16 accounts for about 40% and HPV 18 for an additional 30%.¹⁹⁴ There are many histologic subtypes, which include but are not limited to mucinous (the most common, and subdivided into endocervical, intestinal, signet-ring cell, minimal deviation, and villoglandular variants), endometrioid, adenosquamous, clear cell, serous, and mesonephric types.¹⁶⁶ The myriad of subtypes makes cytologic recognition particularly challenging. Some cases of invasive endocervical adenocarcinoma are cytologically indistinguishable from AIS, but in many cases the diagnosis of an invasive adenocarcinoma can be made or at least suggested: 93% of endocervical adenocarcinomas have either suspicious or positive cytology.¹⁹⁵

CYTOMORPHOLOGY OF ENDOCERVICAL ADENOCARCINOMA:

- tumor diathesis (one half or less of cases)
- large, round nucleus
- prominent nucleolus
- abundant cytoplasm

The cells of *mucinous endocervical adenocarcinomas* are often arranged in sheets. Well-differentiated endocervical mucinous adenocarcinomas are composed of columnar cells with abundant, foamy cytoplasm and a basally located nucleus (Fig. 1.60). They are sometimes difficult to distinguish from reactive endocervical cells (Fig. 1.61). Nuclei are pale or hyperchromatic, and mitoses are sometimes present. In moderately and poorly differentiated tumors there is greater variation in nuclear size and shape, and nucleoli are prominent (Fig. 1.62). A tumor diathesis is present in only about one half of cases¹⁹⁶ (see Fig. 1.60), which contributes to the difficulty in distinguishing AIS from invasive adenocarcinoma.

Adenosquamous carcinoma is composed of malignant squamous and glandular cells arranged in sheets of large pleomorphic cells with abundant dense cytoplasm and prominent macronucleoli. *Clear cell carcinomas* of the endocervix and the vagina are morphologically identical: both are composed of round cells with pale nuclei, prominent nucleoli, and abundant foamy or finely granular cytoplasm.

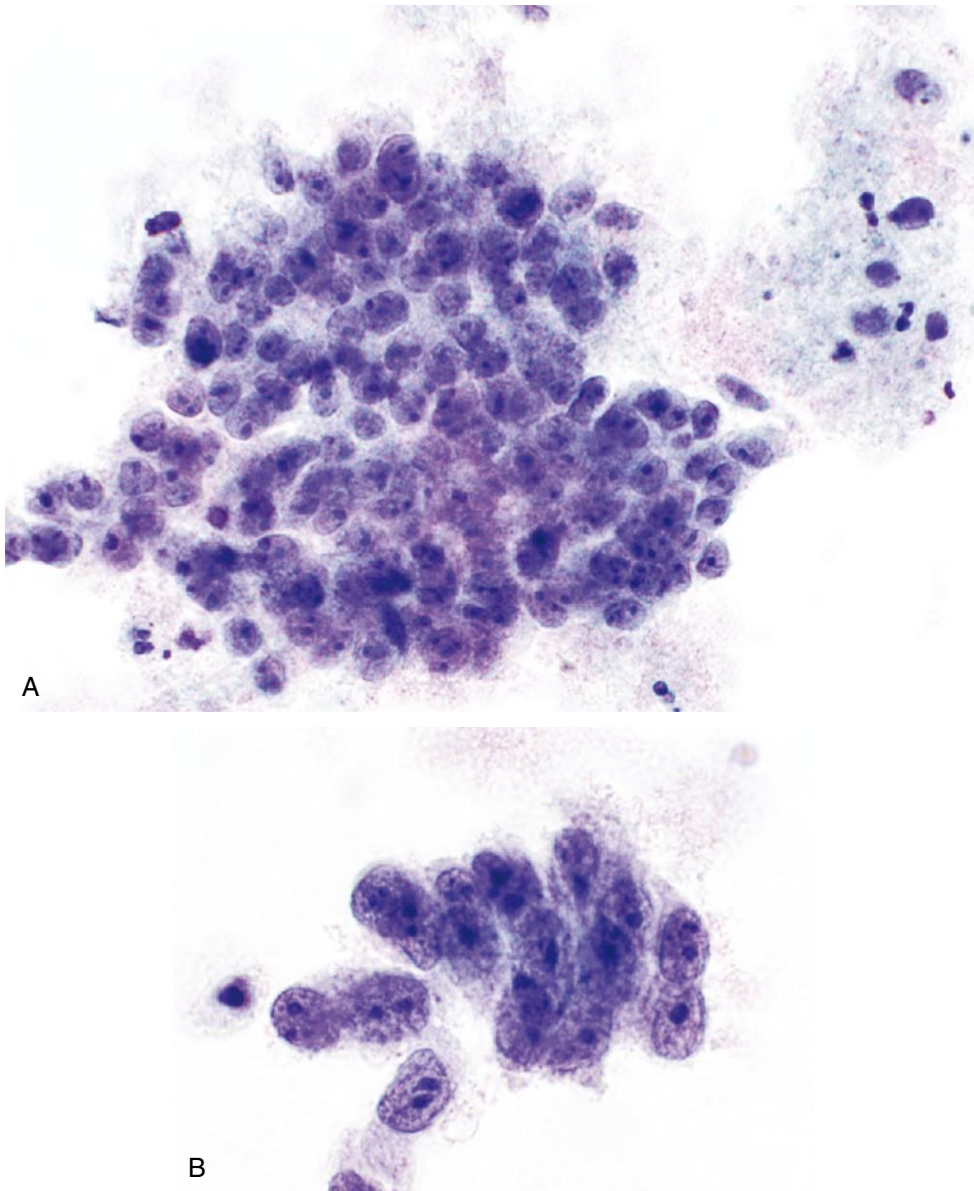


Figure 1.60 Endocervical adenocarcinoma. A, The cells are round rather than elongated as in adenocarcinoma in situ (AIS). They are crowded and hyperchromatic, and a tumor diathesis is present. Tumor diathesis on liquid-based preparations appears as clumps and as a granular ring around groups of malignant cells ("clinging diathesis"). B, High magnification reveals the crowding and large nucleoli.

The rare, extremely well-differentiated tumor known as *minimal deviation adenocarcinoma* (or adenoma malignum) is composed of mucinous glands that show little if any atypia, and yet, if untreated, invade deeply and metastasize. Patients sometimes present with vaginal discharge. In most cases, the neoplastic cells on the Pap test look entirely like normal endocervical cells¹⁹⁷ (Fig. 1.63A). Frequently, even cervical biopsies and endocervical curettages are misinterpreted as benign. A correct diagnosis often requires at least a cone biopsy to appreciate the invasive nature of the lesion (Fig. 1.63B).

Villoglandular adenocarcinomas are rare low-grade neoplasms that rarely if ever metastasize. Cytologically,

they resemble AIS because, like AIS, the cells appear uniform, and crowded, with mild to moderate atypia. Like AIS, strips and rosettes are seen,¹³⁵ and there is no tumor diathesis.¹⁹⁸ Few are diagnosed prospectively as an adenocarcinoma. Most are reported as benign or as "atypical glandular cells."¹⁹⁸

The cytologic features of the rare *adenoid cystic carcinoma* and *mucoepidermoid carcinoma* of the cervix are similar to their counterparts in the salivary gland and elsewhere (see Figs. 10.17, 10.18, 10.20 to 10.22).

Endometrial Adenocarcinoma

Endometrial adenocarcinoma is predominantly a tumor of postmenopausal women, with a peak incidence in

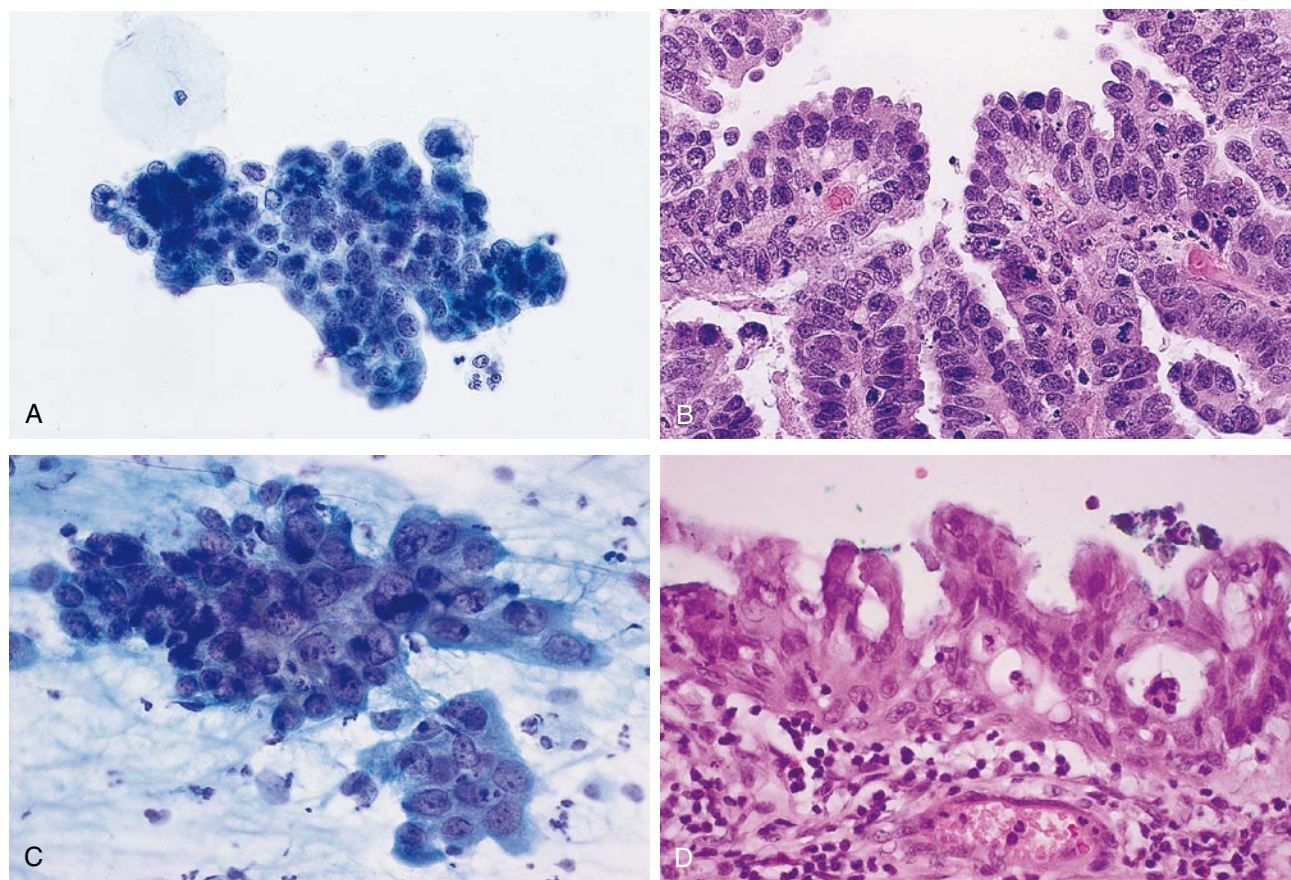


Figure 1.61 Endocervical adenocarcinoma compared to reactive endocervical cells. *A*, Endocervical adenocarcinoma, well differentiated. The cells are enlarged and crowded, but the features are not conclusive for malignancy (note the absence of tumor diathesis). A diagnosis of atypical glandular cells was made. *B*, Histologic sections showed adenocarcinoma. *C*, Reactive endocervical cells. These cells appear similar to those in *A*. *D*, Biopsies in this patient confirmed reactive changes resulting from inflammation.

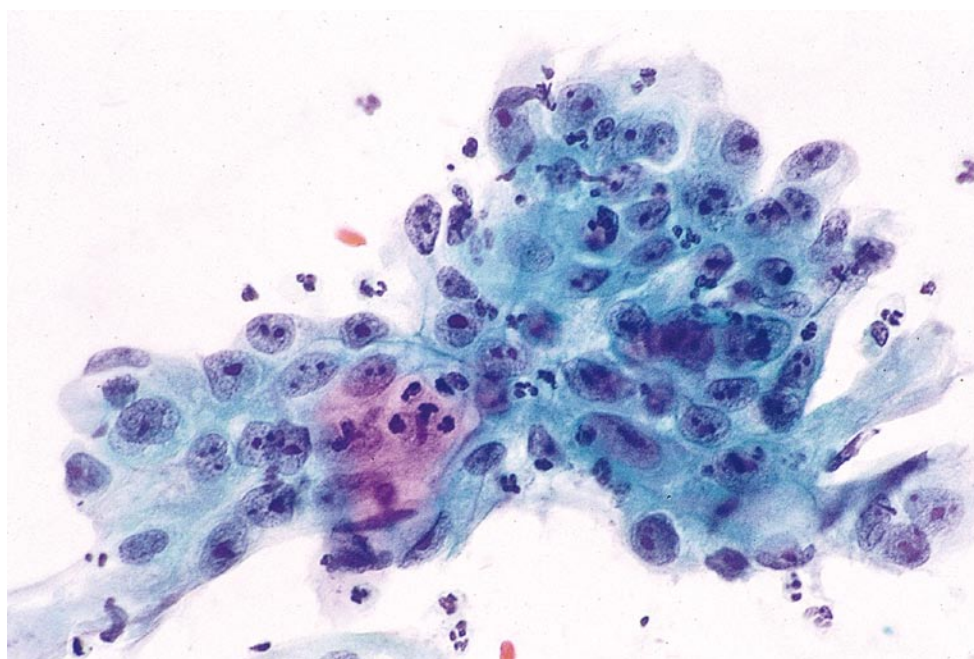


Figure 1.62 Endocervical adenocarcinoma. These malignant cells show variation in nuclear size, with prominent nucleoli and coarsely granular chromatin.

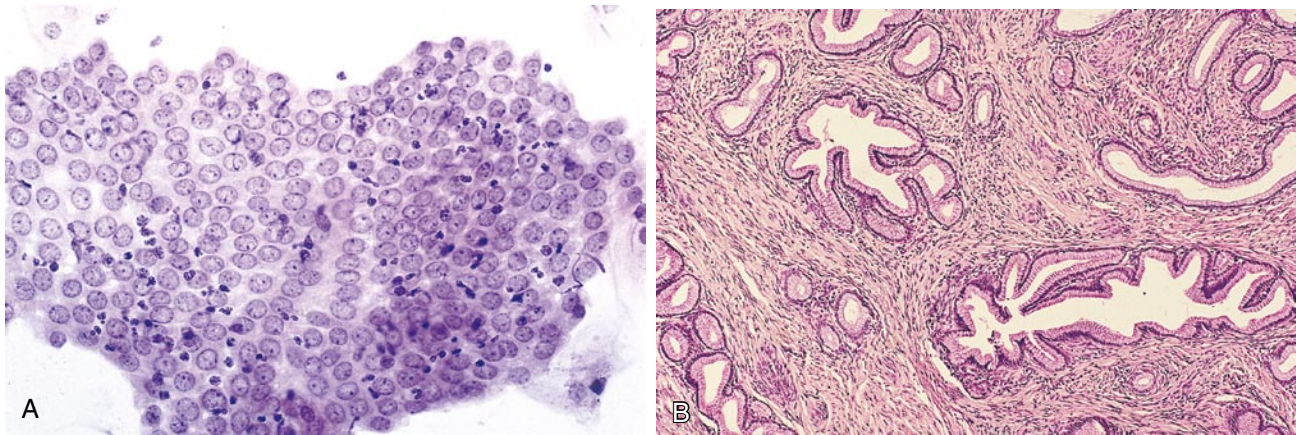


Figure 1.63 Minimal deviation adenocarcinoma. A, The cells are sometimes impossible to distinguish from normal endocervical cells, as in this case. B, A cone biopsy revealed deeply invasive, misshapen neoplastic glands.

women in their late 50s and early 60s; it is rare in women younger than 40. About 90% present with postmenopausal bleeding, but some are asymptomatic. Most endometrial adenocarcinomas are of the endometrioid type. Less common types include serous and clear cell adenocarcinomas, which present at a more advanced stage and have a worse prognosis. The mucinous type of endometrial carcinoma, by contrast, behaves like the endometrioid type.

The Pap test is mainly a screening test for cervical lesions and is not intended for the detection of endometrial lesions.⁸⁵ Nevertheless, the Pap test does fortuitously pick up cells from many endometrial cancers. The cells that exfoliate from high-grade endometrial adenocarcinomas, particularly those of papillary serous or clear cell type, are obviously malignant, and such cases can and are reported as adenocarcinomas (or, if there is doubt, as “atypical endometrial cells”). Cervical Pap cytology is atypical, suspicious, or positive for malignancy in 38% to 90% of endometrial adenocarcinomas.^{199,200}

CYTOMORPHOLOGY OF ENDOMETRIAL ADENOCARCINOMA:

- round cells
- enlarged nucleus
- hyperchromatic
- prominent nucleolus
- scant or abundant vacuolated cytoplasm
- cytoplasmic neutrophils (“bags of polyps”)

In many cases of the endometrioid type of endometrial cancer, the malignant cells are not at all numerous, and only about one third of cases contain a tumor diathesis.²⁰¹ The malignant cells are round, isolated or in groups, and larger and more vacuolated than benign endometrial cells (Fig. 1.64A). Histiocytes frequently accompany the atypical cells, and in some cases outnumber them.

Cells from serous adenocarcinoma of the endometrium are typically large, pleomorphic, and easily recognized as malignant. Numerous bare nuclei in a necrotic

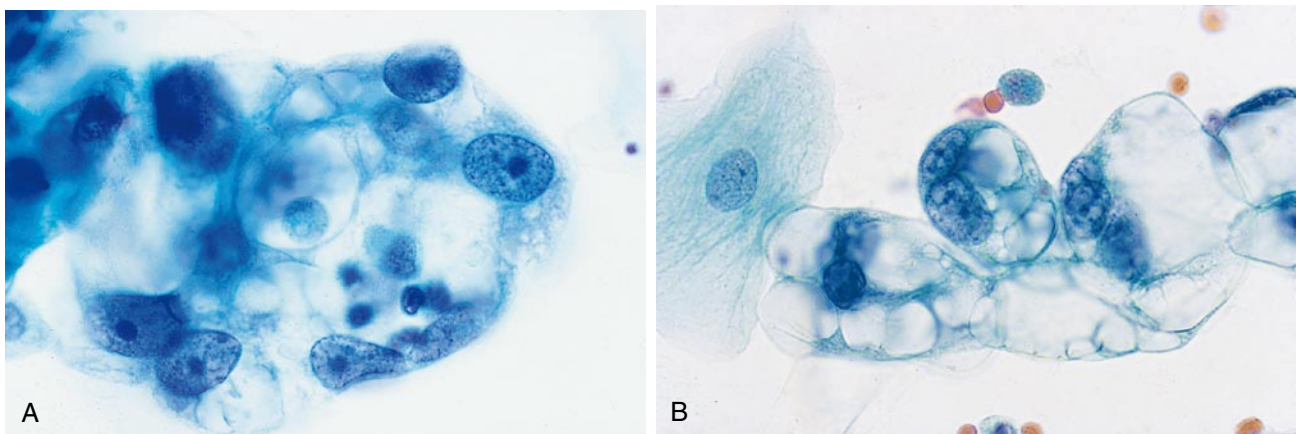


Figure 1.64 Endometrial adenocarcinoma compared to intrauterine device (IUD) effect. A, Endometrial adenocarcinoma, endometrioid type. These malignant cells are large, vacuolated, and associated with neutrophils. B, IUD effect. Benign cells in women with an IUD are indistinguishable morphologically from those of endometrial adenocarcinomas.

background are characteristic. Compared with smears from the endometrioid type, smears from papillary serous adenocarcinomas contain more malignant cells.²⁰¹ Psammoma bodies are present in only 25% of cases.²⁰¹

Pap slides are more likely to contain malignant cells in patients with a serous rather than an endometrioid type of endometrial adenocarcinoma.^{202,203}

Differential Diagnosis of Adenocarcinoma

Because there is significant morphologic overlap between adenocarcinomas of the cervix, endometrium, and other sites, they are considered together.

DIFFERENTIAL DIAGNOSIS OF ADENOCARCINOMA:

- endocervical adenocarcinoma
- endometrial adenocarcinoma
- adenocarcinoma of other sites:
 - vaginal
 - ovarian
 - tubal
 - metastatic
- SQC
- IUD effect
- endocervical polyp atypia
- reactive endocervical cells
- AIS
- pemphigus vulgaris

When adenocarcinoma cells are identified on a Pap slide, the two principal suspects are endocervical and endometrial adenocarcinoma. The age of the patient is helpful: The older the patient, the more likely it is that the tumor has arisen in the endometrium. Morphologic features are also helpful. Endometrial adenocarcinoma cells are rounder

and tend to exfoliate as single cells and smaller clusters, often arranged as spheres, whereas the cells of endocervical adenocarcinomas are more columnar and more commonly shed as sheets of cells. Histiocytes commonly accompany endometrial carcinomas and not endocervical carcinomas. Ultimately, the cytologist can usually only suggest the possibilities, favoring one site over another; the final classification rests on histologic examination.

Adenocarcinoma of the vagina is rare and often associated with a maternal history of DES use during pregnancy.

Adenocarcinomas from the ovaries and fallopian tubes are more commonly associated with psammoma bodies,²⁰⁴ but this is not entirely reliable because endocervical and endometrial cancers sometimes contain them as well.

Nonkeratinizing SQCs resemble endocervical adenocarcinomas. Unless focal keratinization is identified, a definite distinction is not possible. The cells of IUD effect are indistinguishable from those of endometrial adenocarcinoma (Fig. 1.64B). If the woman has an IUD, it is likely that the cells represent IUD effect rather than an adenocarcinoma.

Enlarged, vacuolated cells with engulfed neutrophils (“bags of polyps”) are seen with inflamed endocervical polyps and represent reactive endocervical cells (Fig. 1.65), yet they mimic a similar cell that is characteristic of endometrial carcinoma. Morphologic distinction can be impossible, and knowledge that the patient has an endocervical polyp may be the only clue to correct interpretation.

Reactive endocervical cells, including atypical repair, can mimic adenocarcinomas and vice versa.⁷¹ Reactive cells, paradoxically, often show more marked variation in nuclear size and nucleolar size and shape than adenocarcinomas, which are often deceptively uniform.⁷¹ Reactive cells have thin nuclear membranes

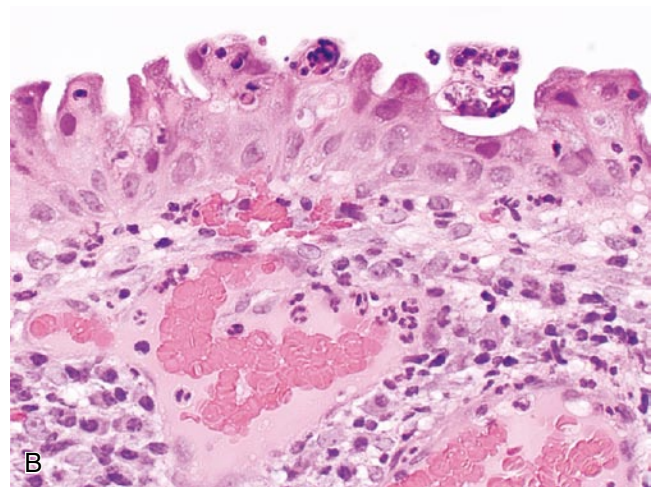
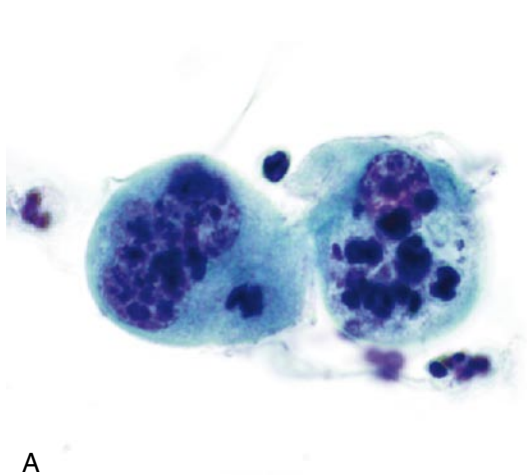


Figure 1.65 Inflamed endocervical polyp mimicking endometrial adenocarcinoma. A, The large vacuolated cells are associated with neutrophils, just like the cells of endometrial adenocarcinoma. B, Histologic sections reveal an acutely inflamed polyp line by reactive endocervical cells infiltrated by polyps.

compared with those of adenocarcinomas, which are often thick and sometimes irregular in contour. Reactive cells form sheets but rarely balls of cells, as is seen with many adenocarcinomas. There are cases, however, where doubt remains; these are diagnosed as “atypical glandular cells.”

The distinction from AIS is problematic and not possible in many cases. If a tumor diathesis is present or the cells are round and have prominent nucleoli, the tumor is more than likely an invasive adenocarcinoma.

Pemphigus vulgaris is a rare blistering disorder that involves mucous membranes, including the cervix. The squamous cells of the cervix lose their squamous morphology and take on a pseudoglandular appearance, with a pale nucleus and prominent nucleolus. The features resemble those of repair except that isolated cells are prominent.²⁰⁵

Atypical Glandular Cells

The category “atypical glandular cells” (AGC) is reserved for cases in which the cellular changes fall between those of a definite benign reactive process and those of an unequivocal AIS or adenocarcinoma. This category, which represents 0.2% to 0.3% of all Pap interpretations,^{87,206} should be used only when the atypia raises the suspicion of AIS or adenocarcinoma; any case that is recognized as clearly benign should be reported as NILM. AGC is subclassified as “atypical endocervical,” “atypical endometrial,” or “not otherwise specified.”

About 30% of patients with AGC have a significant lesion. Although some are AIS (3%) or invasive adenocarcinoma (5%), most of the significant lesions turn out to be CINs (20%).²⁰⁶ This underscores the resemblance of HSIL and AIS. Cell block preparations from the residual LBC sample can be helpful by providing a “histologic” look at the atypical cells.²⁷ Similarly, immunohistochemistry for p63, which highlights squamous but not glandular lesions, can be helpful in selected AGC cases to distinguish between squamous and true glandular lesions.²⁰⁷

Atypical Endocervical Cells

This category includes cases in which an endocervical cell atypia raises the possibility of endocervical AIS or adenocarcinoma, but a benign endocervical reaction like an atypical endocervical repair, pregnancy-related change (e.g., Arias-Stella), endocervical polyp atypia, and microglandular hyperplasia¹²⁸ cannot be excluded (see Fig. 1.61). Depending on the severity of the atypia, one can leave the interpretation of “atypical endocervical cells” unqualified (“not otherwise specified”), or qualify it as “favor neoplastic” if a neoplasm is strongly favored.

DIFFERENTIAL DIAGNOSIS OF ATYPICAL ENDOCERVICAL CELLS:

- reactive endocervical cells
- ASC-H
- HSIL

The differential diagnosis of atypical endocervical cells includes reactive endocervical cells and squamous lesions. If endocervical cells have enlarged nuclei, but the nuclei are round and regular in contour, with finely textured chromatin and prominent nucleoli, they are most likely reactive (see Fig. 1.27B). To qualify as atypical, endocervical cell nuclei should raise the suspicion of AIS, (i.e., they should be elongated, hyperchromatic, and crowded, often with an elevated nuclear-to-cytoplasmic ratio). A common error is mistaking squamous lesions, particularly HSILs, for atypical endocervical cells. Many HSILs have transparent and even vacuolated cytoplasm (see Fig. 1.39). Atypical cells with a rounded contour are more likely to be HSIL than AIS, and for such cases ASC-H is a more appropriate interpretation. The cells of AIS are usually recognizably columnar. For this reason, atypical endocervical cells should be reserved for cells with a recognizably columnar morphology.

Because of the high incidence of significant lesions in women with atypical endocervical cells, colposcopy with endocervical sampling is recommended (Fig. 1.66). For women older than 35 years and younger women with unexplained vaginal bleeding, endometrial sampling should be included.³⁹ If one had not already been obtained, an HPV test at the time of colposcopy is recommended.

If, after colposcopy, no neoplasia is identified histologically for atypical endocervical cells, unqualified, a program of repeat Pap tests at 6-month intervals or a combination of Pap and HPV testing is recommended. If invasive disease is not identified during the initial colposcopic workup and the Pap diagnosis was qualified as “favor neoplasia,” a diagnostic excisional procedure is recommended.³⁹

Atypical Endometrial Cells

Atypical endometrial cells are isolated cells or rounded clusters of cells with an enlarged nucleus and one or more additional features of nuclear atypia (e.g., membrane irregularity, prominence of nucleoli). Cytoplasm is scant or moderately abundant and vacuolated. Such cells are suspicious for endometrial adenocarcinoma, but the quantity of the altered cells or the mild degree of atypia prevents a conclusive for malignancy (Fig. 1.67). Similar changes are known to be caused by endometrial

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Figure 1.66 Management guidelines for women with atypical glandular cells (AGC). The guidelines are different for atypical endometrial cells versus all other subcategories of atypical glandular cells. (Reprinted with the permission of ASCCP © American Society for Colposcopy and Cervical Pathology 2008.)

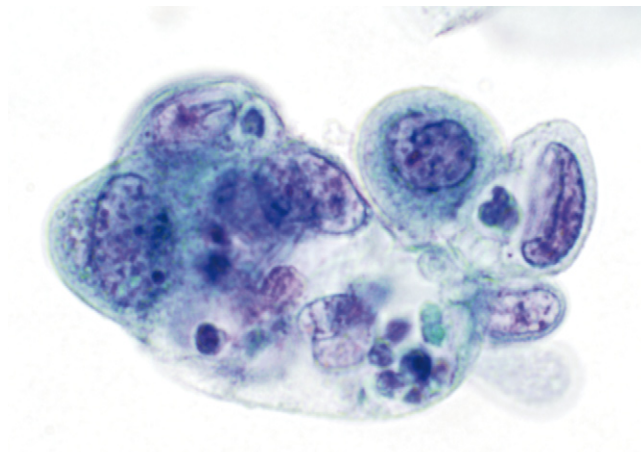


Figure 1.67 Atypical endometrial cells. These cells have enlarged nuclei with slightly irregular contours and some infiltration by neutrophils.

polyps, chronic endometritis, IUDs, and endometrial hyperplasia. The presence of atypical endometrial cells carried a significant risk of cancer,²⁰⁸ and endometrial sampling is recommended.³⁹

OTHER MALIGNANT NEOPLASMS

Small Cell Carcinoma

Tumors that resemble small cell carcinomas of the lung arise in the uterine cervix. Some have concomitant evidence of squamous cell differentiation and are considered variants of poorly differentiated SQC. True small

cell carcinomas, however, are a distinct entity, commonly associated with HPV type 18.²⁰⁹ They are highly aggressive, with a predilection for the early development of distant metastases.

CYTOMORPHOLOGY OF SMALL CELL CARCINOMA:

- clusters of small cells
- hyperchromatic nucleus
- nuclear molding
- scant cytoplasm
- mitoses
- nuclear smearing

Cytologic preparations show clusters of small cells with hyperchromatic nuclei and finely granular chromatin. Cytoplasm is scant. Nuclear molding is present (see Fig. 1.13D), as are mitotic figures. Like their counterparts in the lung, these cells are fragile and show nuclear smearing. Often poorly preserved, the cells are easily confused with menstrual endometrial cells. Nuclear smearing and mitoses, however, are uncommon with endometrial cells and provide a good clue to the diagnosis of a small cell carcinoma.

Malignant Melanoma

Although more common in the vulva, melanomas can arise in the vagina and, even less frequently, the cervix. Vaginal melanomas occur predominantly in the elderly and are aggressive tumors.

CYTOMORPHOLOGY OF MELANOMA:

- isolated cells
- large cells, epithelioid or spindled
- round or oval nucleus
- melanin (not all cases)

The malignant cells are often isolated rather than clustered, and this pattern is helpful in distinguishing them from the more common carcinomas (Fig. 1.68). Tumor cells are large, with a round or oval nucleus and often have a very prominent single nucleolus. Cytoplasm is scant or abundant and in some cases demonstrates the telltale fine, brown granularity of melanin. Melanophages—histiocytes with abundant coarse ingested pigment—may be present.

Malignant Lymphoma

Non-Hodgkin lymphoma frequently involves the cervix and vagina when the disease is advanced. Rarely, it may arise as a primary tumor at these sites.²¹⁰ Cytologic samples are negative if the mucosa is not ulcerated. The tumor cells are larger than small, mature lymphocytes, with a nucleus that is irregular in contour and coarsely granular. The differential diagnosis includes follicular cervicitis (see Fig. 1.16), which is composed of a mixed population of lymphocytes in various stages of maturation, in contrast to many lymphomas, which are composed of a uniform population of atypical lymphoid cells.

Malignant Mixed Mesodermal Tumors

Malignant mixed mesodermal tumors arise much more commonly in the endometrium than in the cervix. Many cases in which the cervix is involved represent extension from an endometrial primary. As with endometrial carcinoma, the most common symptom is vaginal bleeding. The tumors are composed of malignant glands admixed with malignant spindle cells; the latter may show features of stromal sarcoma, leiomyosarcoma, rhabdomyosarcoma, chondrosarcoma, or liposarcoma. Much of the tumor is made up of undifferentiated cells. Smears are often highly cellular and contain malignant glandular or undifferentiated cells with scant cytoplasm. Malignant spindle cells may be present, but are usually a minor component of the specimen.

Metastatic Tumors

Tumors from many sites can metastasize to the cervix or vagina and be detected on smears. Perhaps the most common are stage III or IV adenocarcinomas of the ovary and fallopian tube, which make their way to the cervix and vagina via the endometrial cavity.¹⁹⁵ These tumors are most commonly serous in type and resemble the serous carcinoma of the endometrium described above. Tumors of the ovary and fallopian tube identified on cervical or vaginal smears may give a clean background if large, necrotic tumor implants have not been formed in the cervix.

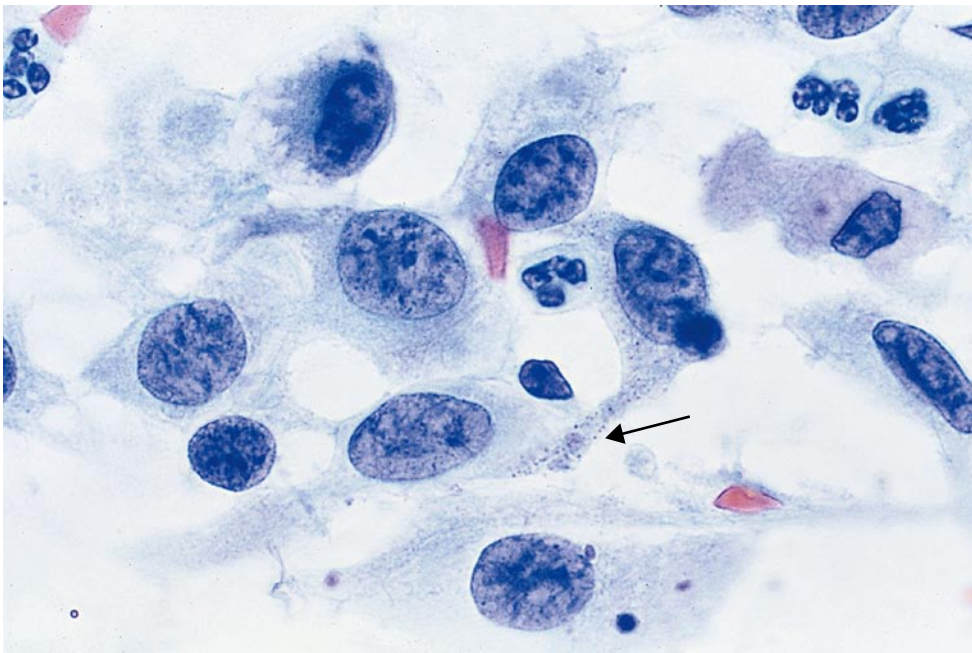


Figure 1.68 Malignant melanoma of the vagina. The malignant spindled and epithelioid cells are dyshesive. There is focal finely granular melanin pigment (arrow).

Psammoma bodies are small, concentrically laminated calcifications that stain dark blue on Papanicolaou stains. They are commonly seen in some tumors of the ovary, fallopian tube, endometrium, and peritoneum, but are extremely rare in routine cervical vaginal smears.²⁰⁴ Their presence should prompt a search for a neoplasm, especially if they are associated with atypical cells²⁰⁴ (Fig. 1.69).

Carcinomas of the colon and rectum can spread directly to the vagina. Tumor cells frequently have a columnar shape with large, hyperchromatic nuclei and

a high nuclear-to-cytoplasmic ratio. Isolated cells may have a signet ring cell appearance. Tumor necrosis may be present.

Carcinomas of the bladder and urethra can also spread to the vagina. Tumor cells are large with hyperchromatic nuclei and without distinguishing features. Clinical correlation is needed for determining the site of origin.

Tumors from distant sites like the breast, kidney, pancreas, and lung can metastasize to the female genital tract. In general, precisely identifying the primary site is impossible without the clinical history and previous biopsy material for comparison.

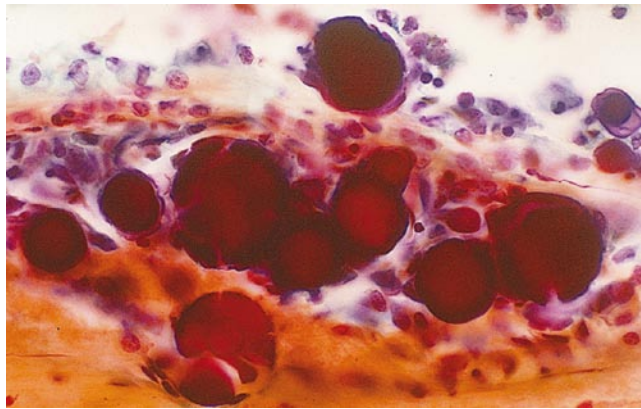


Figure 1.69 Psammoma bodies. These calcific spheres are dark blue or purple and have concentric laminations. They are often fractured, as seen here. These psammoma bodies originated from a borderline serous tumor of the ovary. When cells from ovarian or tubal neoplasms travel through the endometrial cavity, they can be seen on cervical or vaginal Pap samples.

ENDOMETRIAL CELLS IN WOMEN OLDER THAN 40 YEARS OF AGE

Although the Pap test is not employed as a screening test for endometrial cancer, it has been known for decades that benign-appearing endometrial cells in an older woman may be a sign of endometrial cancer. Studies, some of them dating back to the 1970s, have shown that 6% of women with benign-appearing endometrial cells have endometrial carcinoma, and 12% have hyperplasia (Table 1.4).^{211,212} Most of these women come to medical attention because of vaginal bleeding, but 10% to 25% are asymptomatic^{213,214} (Table 1.5). It is not known whether the exfoliated endometrial cells are even neoplastic, or whether they represent just stromal breakdown associated with the neoplasm.

TABLE 1.4 META-ANALYSIS OF BENIGN-APPEARING ENDOMETRIAL CELLS IN POSTMENOPAUSAL WOMEN: PREDICTIVE VALUE FOR ENDOMETRIAL HYPERPLASIA AND CARCINOMA (PRE-BETHESDA 2001)

Authors, Year	Definition of PM	Cases with Biopsy, n	Hyperplasia, n (%)	Cancer, n (%)	Hyperplasia or cancer, n (%)
Cherkis et al., 1988 ^a	≥40	179	23	20	43
Gomez-Fernandez et al., 1999 ^b	?	84	6	6	12
Gondos and King, 1977 ^c	≥40	147	23	2	25
Ng et al., 1974 ^d	≥40	501	52	23	75
Sarode et al., 2001 ^e	>55	81	4	4	8
Yancey et al., 1990 ^f	unknown	74	9	0	9
Zucker et al., 1985 ^g	unknown	23	10	6	16
TOTAL		1089	127 (12%)	61 (6%)	188 (17%)

PM, postmenopausal.

^aCherkis RC, Patten SF, Andrews TJ, et al.: Significance of normal endometrial cells detected by cervical cytology. *Obstet Gynecol* 1988;71:242-244.

^bGomez-Fernandez CR, Ganjei-Azar P, Capote-Dishaw J, Nadji M: Reporting normal endometrial cells in Pap smears: An outcome appraisal. *Gynecol Oncol* 1999;74:381-384.

^cGondos B, King EB: Significance of endometrial cells in cervicovaginal smears. *Ann Clin Lab Sci* 1977;7:486-490.

^dNg ABP, Regan JW, Hawliczek S, Wentz B: Significance of endometrial cells in the detection of endometrial carcinoma and its precursors. *Acta Cytol* 1974;18:356-361.

^eSarode VR, Rader AE, Rose PG, et al.: Significance of cytologically normal endometrial cells in cervical smears from postmenopausal women. *Acta Cytol* 2001;45:153-156.

^fYancey M, Magelssen D, Demazure A, Lee RB: Classification of endometrial cells on cervical cytology. *Obstet Gynecol* 1990;76:1000-1005.

^gZucker PK, Kasdon EJ, Feldstein ML: The validity of Pap smear parameters as predictors of endometrial pathology in menopausal women. *Cancer* 1985;56:2256-2263.

TABLE 1.5 HISTORY OF BLEEDING IN POSTMENOPAUSAL WOMEN WITH ENDOMETRIAL CELLS AND BIOPSY-PROVEN ENDOMETRIAL CANCER

Authors, year	Cancers with endometrial cells on Pap, <i>n</i>	History of bleeding, <i>n</i> (%)	
		Yes	No
Cherkis et al., 1988 ^a	20	15	5 (25%)
Zucker et al., 1985 ^b	18	16	2 (11%)
Gomez-Fernandez et al., 1999 ^c	6	6	0 (0%)
TOTAL	44	37	7 (16%)

^aCherkis RC, Patten SF, Andrews TJ, et al.: Significance of normal endometrial cells detected by cervical cytology. *Obstet Gynecol* 1988;71:242-244.

^bZucker PK, Kasdon EJ, Feldstein ML: The validity of Pap smear parameters as predictors of endometrial pathology in menopausal women. *Cancer* 1985;56:2256-2263.

^cGomez-Fernandez CR, Ganjei-Azar P, Capote-Dishaw J, Nadji M: Reporting normal endometrial cells in Pap smears: An outcome appraisal. *Gynecol Oncol* 1999;74:381-384.

Because of the associated risk, the 1991 Bethesda System recommended that benign-appearing endometrial cells in postmenopausal women should be reported as an epithelial cell abnormality. The recommended terminology was “endometrial cells, cytologically benign, in a postmenopausal woman.” This presented an unanticipated difficulty, however, because menopausal history was not always provided. If the menopausal status was not given, could this diagnosis be made based on age? If so, how old should a woman be for this diagnosis to apply? The median age of final menstrual period is 51 years, but the coefficient of variation is large.²¹⁵

In the 2001 revision of the Bethesda System, the diagnosis is recommended for all women aged 40 and above, irrespective of menstrual status.³⁸ This threshold was selected to optimize sensitivity because cases of endometrial carcinoma have been detected in women between the ages of 40 and 50 who have benign-appearing endometrial cells on their Pap smears.²¹⁶

Whether the risk applies to postmenopausal women on hormone replacement therapy (HRT) is not clear. Data are sparse, but some investigators have found that Pap samples with benign-appearing endometrial cells do identify a small number of asymptomatic women on HRT with endometrial adenocarcinoma and hyperplasia.²¹⁷

It was once believed that histiocytes alone convey an increased risk for endometrial cancer.²¹⁸ This has been widely refuted.^{214,219,220} Thus, only spontaneously exfoliated endometrial cells (Fig. 1.70) are considered significant. Directly abraded endometrium or LUS, like histiocytes, should not be reported under this heading.

Benign-appearing endometrial cells in women over 40 are usually not from a cancer or hyperplasia (see Table 1.4). In most women, they are physiologic (the woman is still cycling, either naturally or because of HRT), or a result of benign endometrial pathology (e.g., an endometrial polyp). For this reason, an endometrial sample is not indicated for all women with this diagnosis. The woman's physician, who knows her

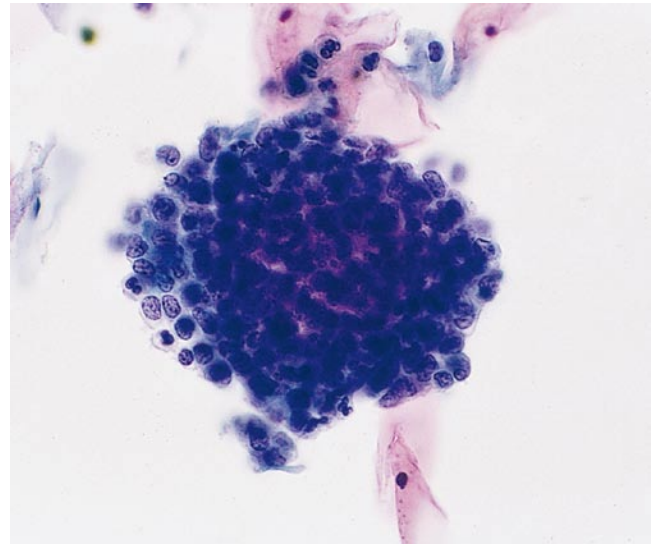


Figure 1.70 Endometrial cells in a woman older than 40 years of age. These cells are indistinguishable from menstrual endometrial cells (see Fig. 1.12).

menstrual and menopausal status, clinical risk factors for endometrial cancer, and whether or not she is on HRT, should use his or her clinical judgment in deciding whether or not to take a histologic endometrial sample. Consensus guidelines recommend that an endometrial sample should be obtained if she is postmenopausal.³⁹

An educational note can be particularly helpful, as in this sample interpretation:

Satisfactory for evaluation.

Endometrial cells, cytologically benign, in a woman older than or equal to 40 years of age.

Negative for SIL.

Note: Endometrial cells after age 40, particularly out of phase or after menopause, may be associated with benign endometrium, hormonal alterations, and less commonly, endometrial abnormalities. Suggest clinical correlation.

TABLE 1.6 META-ANALYSIS OF BENIGN-APPEARING ENDOMETRIAL CELLS IN WOMEN OVER 40: PREDICTIVE VALUE FOR ENDOMETRIAL HYPERPLASIA AND CARCINOMA (POST-BETHESDA 2001)

Authors, year	Cases with biopsy, n	Hyperplasia, n (%)	Cancers, n (%)	Hyperplasia or cancer, n (%)
Browne et al., 2005 ^a	211	1 (0.5)	6 (2.8)*	7 (3.3)
Thrall et al., 2005 ^b	159	9 (5.7)	0	9 (5.7)
Bean et al., 2006 ^c	140	2 (1.4)	0	2 (1.4)
Kapali et al., 2007 ^d	499	4 (0.8)	4 (0.8)	8 (1.6)
TOTAL	1099	16 (1.4)	10 (0.9)	26 (2.4)

*Two women with cancer were premenopausal and asymptomatic.

^aBrowne TJ, Genest DR, Cibas ES: The clinical significance of benign-appearing endometrial cells on a Papanicolaou test in women 40 years or older. *Am J Clin Pathol* 2005;124(6):834-837.

^bThrall MJ, Kjeldahl KS, Savik K, et al.: Significance of benign endometrial cells in Papanicolaou tests from women aged > or = 40 years. *Cancer* 2005;105(4):207-216.

^cBean SM, Connolly K, Roberson J, et al.: Incidence and clinical significance of morphologically benign-appearing endometrial cells in patients age 40 years or older: The impact of the 2001 Bethesda System. *Cancer* 2006;108(1):39-44.

^dKapali M, Agaram NP, Dabbs D, et al.: Routine endometrial sampling of asymptomatic premenopausal women shedding normal endometrial cells in Papanicolaou tests is not cost effective. *Cancer* 2007;111(1):26-33.

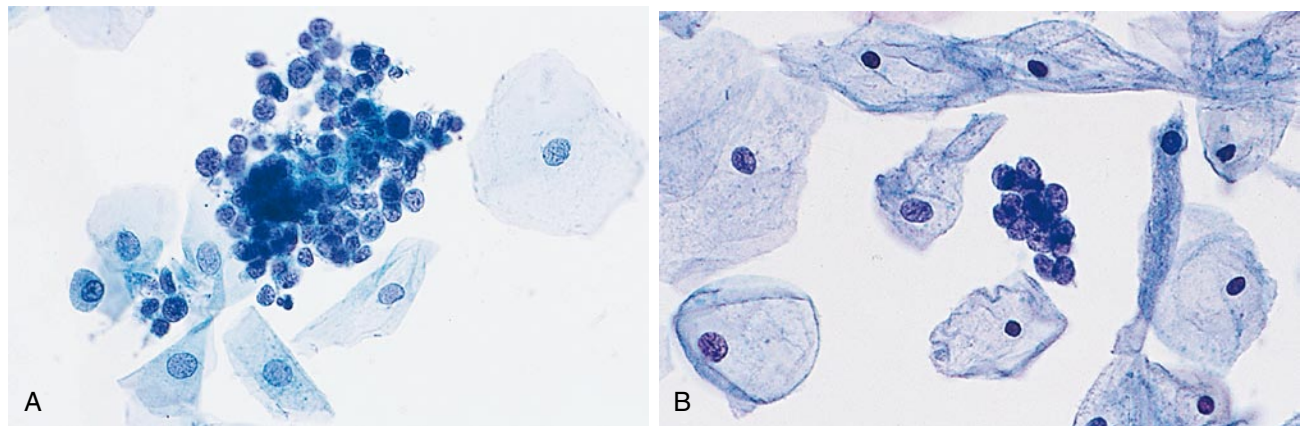


Figure 1.71 Mimics of endometrial cells. A, Follicular cervicitis. Lymphocytes are the same size as endometrial cells, but less tightly clustered. B, Bare squamous cell nuclei. They are about the size of endometrial cells and sometimes aggregate. Cells that lack cytoplasm should not be interpreted as endometrial cells.

In the 2001 Bethesda System, this interpretation is no longer categorized as an epithelial cell abnormality, and because of the small but definite risk of a significant endometrial lesion, neither is it categorized as NILM. This orphan diagnosis, therefore, falls into the general categorization “Other,” a heading some laboratories simply omit from the report, as in the preceding example. Because the primary goal of the Pap test is the identification of squamous precursors, the explicit statement “negative for squamous intraepithelial lesion” is included.

This Pap diagnosis represents 0.5% to 1% of all Pap reports.²²¹⁻²²⁴ The detection of endometrial hyperplasia and cancer since the implementation of the 2001 Bethesda System is shown in Table 1.6.

DIFFERENTIAL DIAGNOSIS OF ENDOMETRIAL CELLS IN WOMEN OVER 40:

- crushed endocervical cells
- follicular cervicitis
- small blue nuclei

A common mimic of endometrial cells in older women is the cluster of crushed, atrophic endocervical cells. They are recognized on the basis of some residual columnar shape. Another is follicular cervicitis (Fig. 1.71A), manifested by lymphoid cell clusters. Lymphoid cells are smaller than exfoliated endometrial cells and less tightly cohesive. Admixed larger, paler dendritic cell nuclei and tingible-body macrophages are typical of follicular cervicitis. Clusters of naked squamous cell nuclei (Fig. 1.71B) are easily mistaken for endometrial cells, but can be identified because they have no cytoplasm. Naked squamous cell nuclei (often called “small blue cells”) are common in postmenopausal women and thus a frequent mimic of endometrial cells. They are seen in 21% of Pap samples from women over the age of 50, and their prevalence is proportional to the woman's age.²²⁵ At one time their presence was associated with tamoxifen, a nonsteroidal estrogen used in the treatment and prevention of breast cancer, but the frequency of small blue nuclei is no higher in these patients than in women who are not taking tamoxifen.²²⁵

REFERENCES

- Janicek MF, Averette HE: Cervical cancer: Prevention, diagnosis, and therapeutics. *CA Cancer J Clin* 2001;51:92-114.
- Jemal A, Siegel R, Ward E, et al.: Cancer statistics, 2007. *CA Cancer J Clin* 2007;57(1):43-66.
- Saslow D, Castle PE, Cox JT, et al.: American Cancer Society Guideline for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. *CA Cancer J Clin* 2007;57(1):7-28.
- WHO meeting: control of cancer of the cervix uteri. *Bull World Health Organ* 1986;64:607-618.
- Papanicolaou GN: New cancer diagnosis. Proceedings: The Third Race Betterment Conference. Battle Creek, Mich, Race Betterment Foundation 1928, pp. 528-534.
- Papanicolaou GN, Traut HF: Diagnosis of Uterine Cancer by Vaginal Smears. New York, The Commonwealth Fund, 1943.
- Papanicolaou GN, Traut HF: The diagnostic value of vaginal smears in carcinoma of the uterus. *Am J Obstet Gynecol* 1941;42:193-206.
- Ayre JE: Selective cytology smear for diagnosis of cancer. *Am J Obstet Gynecol* 1947;53:609-617.
- McSweeney DJ, McKay DG: Uterine cancer: its early detection by simple screening methods. *N Engl J Med* 1948;238:867-870.
- Anderson GH, Boyes DA, Benedet JL, et al.: Organisation and results of the cervical cytology screening programme in British Columbia, 1955-85. *BMJ* 1988;296:975-8.
- Christopherson WM, Scott MA: Trends in mortality from uterine cancer in relation to mass screening. *Acta Cytol* 1977;21:5-9.
- Laara E, Day NE, Hakama M: Trends in mortality from cervical cancer in the Nordic countries: association with organised screening programmes. *Lancet* 1987;1:1247-1249.
- MacGregor JE, Teper S: Mortality from carcinoma of cervix uteri in Britain. *Lancet* 1978;2:774-777.
- Miller AB, Lindsay J, Hill GB: Mortality from cancer of the uterus in Canada and its relationship to screening for cancer of the cervix. *Int J Cancer* 1976;17:602-612.
- Cramer DW: The role of cervical cytology in the declining morbidity and mortality of cervical cancer. *Cancer* 1974;34:2018-2027.
- Clarke EA, Anderson TW: Does screening by "Pap" smears help prevent cervical cancer? A case-control study. *Lancet* 1979;2:1-4.
- Lynge E, Poll P: Incidence of cervical cancer following negative smear: A cohort study from Maribo County, Denmark. *Am J Epidemiol* 1986;124:345-352.
- ACOG practice bulletin. Cervical cytology screening. *Int J Gynaecol Obstet* 2003;83(2):237-247.
- Smith RA, Cokkinides V, Eyre HJ: American Cancer Society guidelines for the early detection of cancer, 2006. *CA Cancer J Clin* 2006;56(1):11-25;quiz 49-50.
- U.S. Preventive Services Task Force. Screening for cervical cancer. Available at www.ahcprgov/clinic/uspstf/uspsscervhtm 2003.
- Leyden WA, Manos MM, Geiger AM, et al.: Cervical cancer in women with comprehensive health care access: attributable factors in the screening process. *J Natl Cancer Inst* 2005;97(9):675-683.
- Cutts FT, Franceschi S, Goldie S, et al.: Human papillomavirus and HPV vaccines: a review. *Bull World Health Organ* 2007;85(9):719-726.
- NCCLS. Papanicolaou Technique; Approved Guideline, 2nd ed. NCCLS document GP15-A2 [ISBN 1-56238-418-X]. Wayne, Pa, NCCLS, 2001.
- Hathaway JK, Pathak PK, Maney R: Is liquid-based pap testing affected by water-based lubricant? *Obstet Gynecol* 2006;107(1):66-70.
- Ronco G, Cuzick J, Pierotti P, et al.: Accuracy of liquid based versus conventional cytology: Overall results of new technologies for cervical cancer screening: randomised controlled trial. *BMJ* 2007;335(7609):28.
- Davey E, Barratt A, Irwig L, et al.: Effect of study design and quality on unsatisfactory rates, cytology classifications, and accuracy in liquid-based versus conventional cervical cytology: A systematic review. *Lancet* 2006;367(9505):122-132.
- Diaz-Rosario LA, Kabawat SE: Cell block preparation by inverted filter sedimentation is useful in the differential diagnosis of atypical glandular cells of undetermined significance in ThinPrep specimens. *Cancer* 2000;90(5):265-272.
- Keyhani-Rofagha S, Vesey-Sheket M: Diagnostic value, feasibility, and validity of preparing cell blocks from fluid-based gynecologic cytology specimens. *Cancer* 2002;96(4):204-209.
- Lee KR, Ashfaq R, Birdsong GG, et al.: Comparison of conventional Papanicolaou smears and a fluid-based, thin-layer system for cervical cancer screening. *Obstet Gynecol* 1997;90:278-287.
- Bolick DR, Hellman DJ: Laboratory implementation and efficacy assessment of the ThinPrep cervical cancer screening system. *Acta Cytol* 1998;42(1):209-213.
- Papillo JL, Zarka MA, St John TL: Evaluation of the ThinPrep Pap test in clinical practice. A seven-month, 16,314-case experience in northern Vermont. *Acta Cytol* 1998;42(1):203-208.
- Diaz-Rosario LA, Kabawat SE: Performance of a fluid-based, thin-layer Papanicolaou smear method in the clinical setting of an independent laboratory and an outpatient screening population in New England. *Arch Pathol Lab Med* 1999;123(9):817-821.
- Guidos BJ, Selvaggi SM: Use of the Thin Prep Pap Test in clinical practice. *Diagn Cytopathol* 1999;20(2):70-73.
- Harkness CB, Theofrastous JP, Ibrahim SN, et al.: Papanicolaou and thin-layer cervical cytology with colposcopic biopsy control. A comparison. *J Reprod Med* 2003;48(9):681-686.
- Limaye A, Connor AJ, Huang X, Luff R: Comparative analysis of conventional Papanicolaou tests and a fluid-based thin-layer method. *Arch Pathol Lab Med* 2003;127(2):200-204.
- Roberts JM, Thurloe JK: Comparative sensitivities of ThinPrep and Papanicolaou smear for adenocarcinoma in situ (AIS) and combined AIS/high-grade squamous intraepithelial lesion (HSIL): Comparison with HSIL. *Cancer* 2007;111(6):482-486.
- Guidos BJ, Selvaggi SM: Detection of endometrial adenocarcinoma with the ThinPrep Pap test. *Diagn Cytopathol* 2000;23(4):260-265.
- Solomon D, Schiffman M, Tarone R: Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: Baseline results from a randomized trial. *J Natl Cancer Inst* 2001;93:293-299.
- Wright TC, Jr, Massad LS, Dunton CJ, et al.: 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests. *Am J Obstet Gynecol* 2007;197(4):346-355.
- Bishop JW, Bigner SH, Colgan TJ, et al.: Multicenter masked evaluation of AutoCyte PREP thin layers with matched conventional smears: Including initial biopsy results. *Acta Cytol* 1998;42:189-197.
- Cibas ES, Alonzo TA, Austin RM, et al.: The MonoPrep Pap Test for the detection of cervical cancer and its precursors. Part I. Results of a multicenter clinical trial. *Am J Clin Pathol* 2008;129(2):193-201.
- Tolles WE, Bostrom RC: Automated screening of cytological smears for cancer: The instrumentation. *Ann NY Acad Sci* 1956;63:1211-1218.
- Husain OAN, Allen RWB, Hawkins EJ, Taylor JE: The Quantimet Cytoscreen and the interactive approach to cancer screening. *J Histochem Cytochem* 1974;22:678-684.

44. Zahniser DJ: The Development of a Fully Automated System for the Prescreening of Cervical Smears. Nijmegen, Netherlands, 1979.
45. Tucker JH, Shippey G: Basic performance tests on the CERVIFIP linear array prescreener. *Anal Quant Cytol* 1983;5:129-137.
46. Tanaka N, Ikeda H, Ueno T, et al.: Automated cytologic screening system (Cybest model 4): An integrated image cytometry system. *Appl Optics* 1987;26:3301-3307.
47. Nordin B: The Development of an Automated Prescreener for the Early Detection of Cervical Cancer: Algorithms and Implementation. Uppsala, 1989.
48. Stenkvist B, Bergstrom R, Brinne U, et al.: Automatic analysis of Papanicolaou smears by digital image processing. *Gynecol Oncol* 1987;27:1-14.
49. Reinhardt ER, Blanz WE, Erhardt R, et al.: Automated classification of cytological specimens based on multistage pattern recognition. *Proceedings of the 6th International Conference on Pattern Recognition*. Munich, 1982, pp. 153-159.
50. Ploem JS, van Driel-Kulker AMJ, Verwoerd NP: Leytas: A cytology screening system using the new modular image analysis computer (MIAC) from Leitz. In Berger G, Ploem JS, Goerttler K (eds): *Clinical Cytometry and Histometry*. London, Academic Press, 1987, pp. 24-35.
51. Tucker J, Stenkvist B: Whatever happened to cervical cytology automation? *Anal Cell Pathol* 1990;2:259-266.
52. Lee JSJ, Kuan L, Seho O, et al.: A feasibility study of the AutoPap System location-guided screening. *Acta Cytol* 1998;42:221-226.
53. Wilbur DC, Prey MU, Miller WM et al.: The AutoPap System for primary screening in cervical cytology. Comparing the results of the prospective intended-use study with routine manual practice. *Acta Cytol* 1998;42:214-222.
54. Cengel KA, Day SJ, Davis-Devine S, et al.: Effectiveness of the SurePath liquid-based Pap test in automated screening and in detection of HSIL. *Diagn Cytopathol* 2003;29(5):250-255.
55. Troni GM, Cariaggi MP, Bulgaresi P, et al.: Reliability of sparing Papanicolaou test conventional reading in cases reported as No Further Review at AutoPap-assisted cytological screening: Survey of 30,658 cases with follow-up cytological screening. *Cancer* 2007;111(2):93-98.
56. Biscotti CV, Dawson AE, Dziura B, et al.: Assisted primary screening using the automated ThinPrep Imaging System. *Am J Clin Pathol* 2005;123(2):281-287.
57. Davey E, d'Assuncao J, Irwig L, et al.: Accuracy of reading liquid based cytology slides using the ThinPrep Imager compared with conventional cytology: Prospective study. *BMJ* 2007;335(7609):31.
58. Dawson AE: Can we change the way we screen? The ThinPrep Imaging System. *Cancer* 2004;102(6):340-344.
59. Schledermann D, Hyldebrandt T, Ejersbo D, Hoelund B: Automated screening versus manual screening: A comparison of the ThinPrep imaging system and manual screening in a time study. *Diagn Cytopathol* 2007;35(6):348-352.
60. Bolger N, Heffron C, Regan I, et al.: Implementation and evaluation of a new automated interactive image analysis system. *Acta Cytol* 2006;50(5):483-491.
61. Dziura B, Quinn S, Richard K: Performance of an imaging system vs. manual screening in the detection of squamous intraepithelial lesions of the uterine cervix. *Acta Cytol* 2006;50(3):309-311.
62. Lozano R: Comparison of computer-assisted and manual screening of cervical cytology. *Gynecol Oncol* 2007;104(1):134-138.
63. Miller FS, Nagel LE, Kenny-Moynihan MB: Implementation of the ThinPrep imaging system in a high-volume metropolitan laboratory. *Diagn Cytopathol* 2007;35(4):213-217.
64. Nanda K, McCrory DC, Myers ER, et al.: Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: A systemic review. *Ann Intern Med* 2000;132:810-819.
65. Attwood ME, Woodman CB, Luesley D, Jordan JA: Previous cytology in patients with invasive carcinoma of the cervix. *Acta Cytol* 1985;29:108-110.
66. Benoit AG, Krepart GV, Lotocki RJ: Results of prior cytologic screening in patients with a diagnosis of Stage I carcinoma of the cervix. *Am J Obstet Gynecol* 1984;148:690-694.
67. Berkowitz RS, Ehrmann RL, Lavizzo-Mourey R, Knapp RC: Invasive cervical carcinoma in young women. *Gynecol Oncol* 1979;8:311-316.
68. Dunn JE, Jr, Schweitzer V: The relationship of cervical cytology to the incidence of invasive cervical cancer and mortality in Alameda County, California. 1960-1974. *Am J Obstet Gynecol* 1981;139:868-876.
69. Fetherston WC: False-negative cytology in invasive cancer of the cervix. *Clin Obstet Gynecol* 1983;26:929-937.
70. Gay JD, Donaldson LD, Goellner JR: False-negative results in cervical cytologic studies. *Acta Cytol* 1985;29:1043-1046.
71. Krane JF, Granter SR, Trask CE, et al.: Papanicolaou smear sensitivity for the detection of adenocarcinoma of the cervix: a study of 49 cases. *Cancer* 2001;93:8-15.
72. Kristensen GB, Skyggebjerg KD, Holund B, et al.: Analysis of cervical smears obtained within three years of the diagnosis of invasive cervical cancer. *Acta Cytol* 1991;35:47-50.
73. Morell ND, Taylor JR, Snyder RN, et al.: False-negative cytology rates in patients in whom invasive cervical cancer subsequently developed. *Obstet Gynecol* 1982;60:41-45.
74. Paterson ME, Peel KR, Joslin CA: Cervical smear histories of 500 women with invasive cervical cancer in Yorkshire. *BMJ* 1984;289:896-898.
75. Rylander E: Negative smears in women developing invasive cervical cancer. *Acta Obstet Gynecol Scand* 1977;56:115-118.
76. van der Graaf Y, Vooijs GP: False negative rate in cervical cytology. *J Clin Pathol* 1987;40:438-442.
77. Mount S, Harmon M, Eltabbakh G, et al.: False positive diagnosis in conventional and liquid-based cervical specimens. *Acta Cytol* 2004;48(3):363-371.
78. Levine PH, Elgert PA, Mittal K: False-positive squamous cell carcinoma in cervical smears: Cytologic-histologic correlation in 19 cases. *Diagn Cytopathol* 2003;28(1):23-27.
79. Stoler MH, Schiffman M: Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL triage study. *JAMA* 2001;285:1500-1505.
80. Bethesda System. Web Atlas. Available at <http://nih.techriver.net/listing.php?histo=1>.
81. Maguire NC: Current use of the Papanicolaou class system in gynecologic cytology. *Diagn Cytopathol* 1988;4:169-176.
82. Richart RM, Barron BA: A follow-up study of patients with cervical dysplasia. *Am J Obstet Gynecol* 1969;105:386-393.
83. Melnikow J, Nuovo J, Willan AR, et al.: Natural history of cervical squamous intraepithelial lesions: A meta-analysis. *Obstet Gynecol* 1998;92(4 Pt 2):727-735.
84. National Cancer Institute Workshop: The 1988 Bethesda System for reporting cervical/vaginal cytologic diagnoses. *JAMA* 1989;262:931-934.
85. Solomon D, Davey D, Kurman R, et al.: The 2001 Bethesda System: Terminology for reporting results of cervical cytology. *JAMA* 2002;287:2114-2119.
86. Solomon D, Nayar R (eds): *The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria, and Explanatory Notes*, 2nd ed. New York, Springer, 2004.
87. Davey DD, Neal MH, Wilbur DC, et al.: Bethesda 2001 implementation and reporting rates: 2003 practices of

- participants in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. *Arch Pathol Lab Med* 2004;128(11):1224-1229.
88. Kurman RJ, Solomon D: The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses: Definitions, Criteria, and Explanatory Notes for Terminology and Specimen Adequacy. New York, Springer-Verlag, 1994.
 89. Renshaw AA, Friedman MM, Rahemtulla A, et al.: Accuracy and reproducibility of estimating the adequacy of the squamous component of cervicovaginal smears. *Am J Clin Pathol* 1999;111:38-42.
 90. Geyer JW, Carrico C: Cellular constitution of Autocyte PREP cervicovaginal samples with biopsy-confirmed HSIL [Abstract]. *Acta Cytol* 2000;44:505.
 91. Hutchinson ML, Isenstein LM, Goodman A, et al.: Homogeneous sampling accounts for the increased diagnostic accuracy using the ThinPrep processor. *Am J Clin Pathol* 1994;101:215-219.
 92. Bentz J, Rowe LR, Gopez EV, Marshall CJ: The unsatisfactory ThinPrep Pap test: Missed opportunity for disease detection? *Am J Clin Pathol* 2002;117:457-463.
 93. Islam S, West AM, Saboorian MH, Ashfaq R: Reprocessing unsatisfactory ThinPrep Papanicolaou test specimens increases sample adequacy and detection of significant cervicovaginal lesions. *Cancer* 2004;102(2):67-73.
 94. Birdsong GG: Pap smear adequacy: Is our understanding satisfactory ... or limited? *Diagn Cytopathol* 2001;24:79-81.
 95. Mauney M, Eide D, Sotham J: Rates of condyloma and dysplasia in Papanicolaou smears with and without endocervical cells. *Diagn Cytopathol* 1990;6:18-21.
 96. Mintzer MP, Curtis P, Resnick JC, Morrell D: The effect of the quality of Papanicolaou smears on the detection of cytologic abnormalities. *Cancer* 1999;87:113-117.
 97. Vooijs PG, Elias A, van der Graaf Y, Veling S: Relationship between the diagnosis of epithelial abnormalities and the composition of cervical smears. *Acta Cytol* 1985;29:323-328.
 98. Mitchell H, Medley G: Differences between Papanicolaou smears with correct and incorrect diagnoses. *Cytopathology* 1995;6:368-375.
 99. O'Sullivan JP, A'Hern RP, Chapman PA, et al.: A case-control study of true-positive versus false-negative cervical smears in women with cervical intraepithelial neoplasia (CIN) III. *Cytopathology* 1998;9:155-161.
 100. Bos AB, van Bellegooijen M, van den Akker-van Marle E: Endocervical status is not predictive of the incidence of cervical cancer in the years after negative smears. *Am J Clin Pathol* 2001;115:851-855.
 101. Kivlahan C, Ingram E: Papanicolaou smears without endocervical cells: Are they inadequate? *Acta Cytol* 1986;30:258-260.
 102. Mitchell H: Longitudinal analysis of histologic high-grade disease after negative cervical cytology according to endocervical status. *Cancer* 2001;93:237-240.
 103. Weir MM, Bell DA: Transitional cell metaplasia of the cervix: A newly described entity in cervicovaginal smears. *Diagn Cytopathol* 1998;18:222-226.
 104. Jonasson JG, Wang HH, Antonioli DA, Ducatman BS: Tubal metaplasia of the uterine cervix: A prevalence study in patients with gynecologic pathologic findings. *Int J Gynecol Pathol* 1992;11:89-95.
 105. Vooijs GP, van der Graaf Y, Vooijs MA: The presence of endometrial cells in cervical smears in relation to the day of the menstrual cycle and the method of contraception. *Acta Cytol* 1987;31:427-433.
 106. Chang BS, Pinkus GS, Cibas ES: Exfoliated endometrial cell clusters in cervical cytologic preparations are derived from endometrial stroma and glands. *Am J Clin Pathol* 2006;125(1):77-81.
 107. Gondos B, King EB: Significance of endometrial cells in cervicovaginal smears. *Ann Clin Lab Sci* 1977;7:486-490.
 108. Yancey M, Magelssen D, Demareuz A, Lee RB: Classification of endometrial cells on cervical cytology. *Obstet Gynecol* 1990;76:1000-1005.
 109. de Peralta-Venturino MN, Purslow J, Kini SR: Endometrial cells of the "lower uterine segment" (LUS) in cervical smears obtained by endocervical brushings: A source of potential diagnostic pitfall. *Diagn Cytopathol* 1995;12:263-271.
 110. Lee KR: Atypical glandular cells in cervical smears from women who have undergone cone biopsy: A potential diagnostic pitfall. *Acta Cytol* 1993;37:705-709.
 111. Fiorella RM, Cheng J, Kragel PJ: Papanicolaou smears in pregnancy: Positivity of exfoliated cells for human chorionic gonadotropin and human placental lactogen. *Acta Cytol* 1993;37:451-456.
 112. Schnadig VJ, Davie KD, Shafer SK, et al.: The cytologist and bacterioses of the vaginal-endocervical area: Clues, commas and confusion. *Acta Cytol* 1989;33:287-297.
 113. Amsel R, Totten PA, Spiegel CA, et al.: Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983;74(1):14-22.
 114. Discacciati MG, Simoes JA, Amaral RG, et al.: Presence of 20% or more clue cells: An accurate criterion for the diagnosis of bacterial vaginosis in Papanicolaou cervical smears. *Diagn Cytopathol* 2006;34(4):272-276.
 115. Saslow D, Runowicz CD, Solomon D, et al.: American Cancer Society guideline for the early detection of cervical neoplasia and cancer. *CA Cancer J Clin* 2002;52:342-262.
 116. Petrin D, Delgaty K, Bhatt R, Garber G: Clinical and microbiological aspects of *Trichomonas vaginalis*. *Clin Microbiol Rev* 1998;11(2):300-317.
 117. Fiorino AS: Intrauterine contraceptive device-associated actinomycotic abscess and *Actinomyces* detection on cervical smear. *Obstet Gynecol* 1996;87:142-149.
 118. Westhoff C: IUDs and colonization or infection with *Actinomyces*. *Contraception* 2007;75(6 Suppl):S48-S50.
 119. Gideon K, Zaharopoulos P: Cytomegalovirus endocervicitis diagnosed by cervical smear. *Diagn Cytopathol* 1991;7:625-627.
 120. Hunt JL, Baloch Z, Judkins A, et al.: Unique cytomegalovirus intracytoplasmic inclusions in ectocervical cells on a cervical/endocervical smear. *Diagn Cytopathol* 1998;18:110-112.
 121. Bernal JN, Martinez MA, Dabancens A: Evaluation of proposed cytomorphologic criteria for the diagnosis of *Chlamydia trachomatis* in Papanicolaou smear. *Acta Cytol* 1989;33:309-313.
 122. Arroyo G, Elgueta R: Squamous cell carcinoma associated with amoebic cervicitis: Report of a case. *Acta Cytol* 1989;33:301-304.
 123. deMoraes-Ruehsen M, McNeill RE, Frost JK, et al.: Amebae resembling *Entamoeba gingivalis* in the genital tracts of IUD users. *Acta Cytol* 1980;24:413-420.
 124. Chandra M: Cervical smear with intracellular organisms from a case of granuloma venereum (donovanosis) [Letter]. *Acta Cytol* 1991;35:143-145.
 125. Kapila K, Verma K: Intracellular bacilli in vaginal smears in a case of malacoplakia of the uterine cervix [Letter]. *Acta Cytol* 1989;33:410-411.
 126. Cibas ES, Browne TJ, Bassichis MH, Lee KR: Enlarged squamous cell nuclei in cervical cytologic specimens from perimenopausal women ("PM Cells"): A cause of ASC overdiagnosis. *Am J Clin Pathol* 2005;124(1):58-61.
 127. Benoit JL, Kini SR: "Arias-Stella reaction"-like changes in endocervical glandular epithelium in cervical smears during pregnancy and postpartum states—A potential diagnostic pitfall. *Diagn Cytopathol* 1996;14:349-355.

128. Yahr LJ, Lee KR: Cytologic findings in microglandular hyperplasia of the cervix. *Diagn Cytopathol* 1991;7:248-251.
129. Fornari ML: Cellular changes in the glandular epithelium of patients using IUCD—A source of cytologic error. *Acta Cytol* 1974;18:341-343.
130. Gupta PK, Burroughs F, Luff RD, et al.: Epithelial atypias associated with intrauterine contraceptive devices (IUD). *Acta Cytol* 1978;22:286-291.
131. Tambouret R, Pitman MB, Bell DA: Benign glandular cells in posthysterectomy vaginal smears. *Acta Cytol* 1998;42:1403-1408.
132. Novotny DB, Maygarden SJ, Johnson DE, Frable WJ: Tubal metaplasia: A frequent potential pitfall in the cytologic diagnosis of endocervical glandular dysplasia on cervical smears. *Acta Cytol* 1992;36:1-10.
133. Young RH, Scully RE: Minimal-deviation endometrioid adenocarcinoma of the uterine cervix: A report of five cases of a distinct neoplasm that may be misinterpreted as benign. *Am J Surg Pathol* 1993;17:660-665.
134. O'Connell F, Cibas ES: Cytologic features of ciliated adenocarcinoma of the cervix: A case report. *Acta Cytol* 2005;49(2):187-910.
135. Chang AR: Carcinoma in situ of the cervix and its malignant potential: A lesson from New Zealand. *Cytopathology* 1990;1:321-328.
136. Koss LG, Stewart FW, Foote FW, et al.: Some histological aspects of behavior of epidermoid carcinoma in situ and related lesions of the uterine cervix: A long-term prospective study. *Cancer* 1963;16:1160-1211.
137. Walboomers JMM, Jacobs MV, Manos MM, et al.: Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999;189:12-19.
138. Ayre JE: The vaginal smear: "Precancer" cell studies using a modified technique. *Am J Obstet Gynecol* 1949;58:1205-1219.
139. Papanicolaou GN: *Atlas of Exfoliative Cytology*. Cambridge, Mass, Harvard University Press, 1954.
140. Koss LG, Durfee GR: Unusual patterns of squamous epithelium of the uterine cervix: Cytologic and pathologic study of koilocytotic atypia. *Ann NY Acad Sci* 1956;63:1235-1261.
141. Meisels A, Fortin R: Condylomatous lesions of the cervix and vagina: I. Cytologic pattern. *Acta Cytol* 1976;20:505-509.
142. Puroila E, Savia E: Cytology of gynecologic condyloma acuminatum. *Acta Cytol* 1977;21:26-31.
143. Hills E, Laverty CR: Electron microscopic detection of papilloma virus particles in selected koilocytotic cells in a routine cervical smear. *Acta Cytol* 1979;23:53-56.
144. Menezes G, Euscher E, Schwartz B, et al.: Utility of the in situ detection of HPV in Pap smears diagnosed as within normal limits. *Acta Cytol* 2001;45:919-926.
145. Bonfiglio TA, Stoler MH: Human papillomavirus and cancer of the uterine cervix. *Hum Pathol* 1988;19:621-622.
146. Dyson N, Howley PM, Munger K, Harlow E: The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 1989;243:934-936.
147. Matlashewski G, Schneider J, Banks L, et al.: Human papillomavirus type 16 DNA cooperates with activated ras in transforming primary cells. *EMBO J* 1987;6:1741-1746.
148. McCance DJ, Kopan R, Fuchs E, Laimans LA: Human papillomavirus type 16 alters human epithelial cell differentiation in vitro. *Proc Natl Acad Sci USA* 1988;85:7169-7173.
149. Schiffman M, Castle PE, Jeronimo J, et al.: Human papillomavirus and cervical cancer. *Lancet* 2007;370(9590):890-907.
150. Thomison J, 3rd, Thomas LK, Shroyer KR: Human papillomavirus: Molecular and cytologic/histologic aspects related to cervical intraepithelial neoplasia and carcinoma. *Hum Pathol* 2008;39(2):154-166.
151. Carter JJ, Koutsky LA, Hughes JB, et al.: Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis* 2000;181(6):1911-1919.
152. Lee KR, Minter LJ, Crum CP: Koilocytotic atypia in Papanicolaou smears. *Cancer* 1997;81:10-15.
153. Sherman ME, Schiffman MH, Erozan YS, et al.: The Bethesda System: A proposal for reporting abnormal cervical smears based on the reproducibility of cytopathologic diagnoses. *Arch Pathol Lab Med* 1992;116:1155-1158.
154. Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial. Lesions Triage Study (ALTS) Group: Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: Baseline data from a randomized trial. *J Natl Cancer Inst* 2000;92:397-402.
155. Jones BA, Novis DA: Cervical biopsy-cytology correlation. A College of American Pathologists Q-Probes study of 22,439 correlations in 348 laboratories. *Arch Pathol Lab Med* 1996;120:523-531.
156. Wright TC, Jr, Massad LS, Dunton CJ, et al.: 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. *Am J Obstet Gynecol* 2007;197(4):340-345.
157. Patten SE: Diseases of the uterine cervix. In Keebler CM, Somrak TM (eds): *The Manual of Cytopathology*, 7th ed. Chicago, American Society of Clinical Pathologists 1993, pp. 106-107.
158. McGrath CM, Kurtis JD, Yu GH: Evaluation of mild-to-moderate dysplasia on cervical-endocervical (Pap) smear: A subgroup of patients who bridge LSIL and HSIL. *Diagn Cytopathol* 2000;23:245-248.
159. Faquin WC, Brown FB, Krane JF, et al.: Extensively keratinized squamous intraepithelial lesions of the cervix are difficult to grade. *Am J Clin Pathol* 2001;115:80-84.
160. Nasser SM, Cibas ES, Crum CP, Faquin WC: The significance of the Papanicolaou smear diagnosis of low-grade squamous intraepithelial lesion cannot exclude high-grade squamous intraepithelial lesion. *Cancer* 2003;99(5):272-276.
161. Elsheikh TM, Kirkpatrick JL, Wu HH: The significance of "low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion" as a distinct squamous abnormality category in Papanicolaou tests. *Cancer* 2006;108(5):277-281.
162. Owens CL, Moats DR, Burroughs FH, Gustafson KS: "Low-grade squamous intraepithelial lesion, cannot exclude high-grade squamous intraepithelial lesion" is a distinct cytologic category: Histologic outcomes and HPV prevalence. *Am J Clin Pathol* 2007;128(3):398-403.
163. Shidham VB, Kumar N, Narayan R, Brotzman GL: Should LSIL with ASC-H (LSIL-H) in cervical smears be an independent category? A study on SurePath specimens with review of literature. *Cytojournal* 2007;4:7.
164. Wang SS, Sherman ME, Hildesheim A, et al.: Cervical adenocarcinoma and squamous cell carcinoma incidence trends among white women and black women in the United States for 1976-2000. *Cancer* 2004;100(5):1035-1044.
165. Clifford GM, Smith JS, Plummer M, et al.: Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 2003;88(1):63-73.
166. Tavassoli FA, Devilee P (eds): *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs*. Lyon, France, IARC Press, 2003.
167. Rushing L, Cibas ES: The frequency of tumor diathesis in smears from women with squamous cell carcinoma of the cervix. *Acta Cytol* 1997;41:781-785.

168. Wilbur DC, Maurer S, Smith NJ: Behçet's disease in a vaginal smear: report of a case with cytologic features and their distinction from squamous cell carcinoma. *Acta Cytol* 1993;37:525-530.
169. Dvoretzky PM, Bonfiglio TA, Patten SF, Helmkamp BF: Pemphigus vulgaris and microinvasive squamous cell carcinoma of the uterine cervix. *Acta Cytol* 1985;29:403-410.
170. Long HJ, 3rd, Laack NN, Gostout BS: Prevention, diagnosis, and treatment of cervical cancer. *Mayo Clin Proc* 2007;82(12):1566-1574.
171. Pitman MB, Cibas ES, Powers CN, et al.: Reducing or eliminating the category of atypical squamous cells of undetermined significance decreases the diagnostic accuracy of the Papanicolaou smear. *Cancer* 2002;96:128-134.
172. Kinney WK, Manos MM, Hurley LB, Ransley JE: Where's the high-grade cervical neoplasia? The importance of minimally abnormal Papanicolaou diagnoses. *Obstet Gynecol* 1998;91:973-976.
173. Nascimento AF, Cibas ES: The ASC/SIL ratio for cytopathologists as a quality control measure: A follow-up study. *Am J Clin Pathol* 2007;128(4):653-656.
174. Juskevicius R, Zou KH, Cibas ES: An analysis of factors that influence the ASCUS/SIL ratio of pathologists. *Am J Clin Pathol* 2001;116:331-335.
175. Collins LC, Wang HH, Abu-Jawdeh GM: Qualifiers of atypical squamous cells of undetermined significance help in patient management. *Mod Pathol* 1996;9:677-681.
176. Genest DR, Dean B, Lee KR, et al.: Qualifying the cytologic diagnosis of "atypical squamous cells of undetermined significance" affects the predictive value of a squamous intraepithelial lesion on subsequent biopsy. *Arch Pathol Lab Med* 1998;122:338-341.
177. Lachman ME, Cavallo-Calvanese C: Quantification of atypical squamous cells of undetermined significance in an independent laboratory: is it useful or significant? *Am J Obstet Gynecol* 1998;179:421-429.
178. Schooland M, Sterrett GF, Knowles SAS, et al.: The "inconclusive-possible high-grade epithelial abnormality" category in Papanicolaou smear reporting. *Cancer* 1998;84:208-217.
179. Wright TC, Cox JT, Massad LS, et al.: 2001 consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA* 2002;287:2120-2129.
180. Symmans F, Mechanic L, MacConnell P, et al.: Correlation of cervical cytology and human papillomavirus DNA detection in post-menopausal women. *Int J Gynecol Pathol* 1992;11:204-209.
181. Kaminski PF, Sorosky JI, Wheelock JB, Stevens CW: The significance of atypical cervical cytology in an older population. *Obstet Gynecol* 1989;73:13-15.
182. Sherman ME, Castle PE, Solomon D: Cervical cytology of atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion (ASC-H): characteristics and histologic outcomes. *Cancer* 2006;108(5):298-305.
183. Boon ME, Baak JP, Kurver PJ, et al.: Adenocarcinoma in situ of the cervix: An underdiagnosed lesion. *Cancer* 1981;48:768-773.
184. Friedell GH, McKay KD: Adenocarcinoma in situ of the endocervix. *Cancer* 1953;6(5):887-897.
185. Krumins I, Young Q, Pacey F, et al.: The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri. *Acta Cytol* 1977;21(2):320-329.
186. Bousfield L, Pacey F, Young Q, et al.: Expanded cytologic criteria for the diagnosis of adenocarcinoma in situ of the cervix and related lesions. *Acta Cytol* 1980;24(4):283-296.
187. Ayer B, Pacey F, Greenberg M, Bousfield L: The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri and related lesions: I. Adenocarcinoma in situ. *Acta Cytol* 1987;31:397-411.
188. Biscotti CV, Gero MA, Toddy SM, et al.: Endocervical adenocarcinoma in situ: an analysis of cellular features. *Diagn Cytopathol* 1997;17:326-332.
189. Lee KR, Manna EA, Jones MA: Comparative cytologic features of adenocarcinoma in situ of the uterine cervix. *Acta Cytol* 1991;35:117-126.
190. Biscotti CV, Hart WR: Apoptotic bodies: a consistent morphologic feature of endocervical adenocarcinoma in situ. *Am J Surg Pathol* 1998;22:434-439.
191. Lee KR, Minter LJ, Granter SR: Papanicolaou smear sensitivity for adenocarcinoma in situ of the cervix: A study of 34 cases. *Am J Clin Pathol* 1997;107:30-35.
192. Pacey F, Ayer B, Greenberg M: The cytologic diagnosis of adenocarcinoma in situ of the cervix uteri and related lesions: III. Pitfalls in diagnosis. *Acta Cytol* 1988;32:325-330.
193. Lee KR, Genest DR, Minter LJ, et al.: Adenocarcinoma in situ in cervical smears with a small cell (endometrioid) pattern: Distinction from cells directly sampled from the upper endocervical canal or lower segment of the endometrium. *Am J Clin Pathol* 1998;109:738-742.
194. Castellsague X, Diaz M, de Sanjose S, et al.: Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. *J Natl Cancer Inst* 2006;98(5):303-315.
195. Sasagawa M, Nishino K, Honma S, et al.: Origin of adenocarcinoma cells observed on cervical cytology. *Acta Cytol* 2003;47(3):410-414.
196. DiTomasso JP, Ramzy I, Mody DR: Glandular lesions of the cervix: validity of cytologic criteria used to differentiate reactive changes, intraepithelial lesions, and adenocarcinoma. *Acta Cytol* 1996;40:1127-1135.
197. Granter SR, Lee KL: Cytologic findings in minimal deviation adenocarcinoma (adenoma malignum) of the cervix: a report of seven cases. *Am J Clin Pathol* 1996;105:327-333.
198. Ballo MS, Silverberg SG, Sidawy MK: Cytologic features of well-differentiated villoglandular adenocarcinoma of the cervix. *Acta Cytol* 1996;40:536-540.
199. Zhou J, Tomashefski JE, Jr, Khiyami A: ThinPrep Pap tests in patients with endometrial cancer: A histo-cytological correlation. *Diagn Cytopathol* 2007;35(7):448-453.
200. Thrall M, Kjeldahl K, Gulbahce HE, Pambuccian SE: Liquid-based Papanicolaou test (SurePath) interpretations before histologic diagnosis of endometrial hyperplasias and carcinomas: Study of 272 cases classified by the 2001 Bethesda system. *Cancer* 2007;111(4):217-223.
201. Wright CA, Leiman G, Burgess SM: The cytomorphology of papillary serous carcinoma of the endometrium in cervical smears. *Cancer* 1999;87:12-18.
202. Kuebler D, Nikrui N, Bell D: Cytologic features of endometrial papillary serous carcinoma. *Acta Cytol* 1989;33:120-126.
203. Todo Y, Minobe S, Okamoto K, et al.: Cytological features of cervical smears in serous adenocarcinoma of the endometrium. *Jpn J Clin Oncol* 2003;33(12):636-641.
204. Kern SB: Prevalence of psammoma bodies in Papanicolaou-stained cervicovaginal smears. *Acta Cytol* 1991;35:81-88.
205. Wright C, Pippingas A, Grayson W, Leiman G: Pemphigus vulgaris of the uterine cervix revisited: Case report and review of the literature. *Diagn Cytopathol* 2000;22:304-307.
206. Schnatz PF, Guile M, O'Sullivan DM, Sorosky JI: Clinical significance of atypical glandular cells on cervical cytology. *Obstet Gynecol* 2006;107(3):701-708.
207. Garcia MT, Acar BC, Jorda M, et al.: Use of p63 for distinction of glandular versus squamous lesions in cervicovaginal specimens. *Cancer* 2007;111(1):54-57.
208. Cherkis RC, Patten SF, Dickinson JC, Dekanich AS: Significance of atypical endometrial cells detected by cervical cytology. *Obstet Gynecol* 1987;69:786-789.

209. Stoler MH, Mills SE, Gersell DJ, Walker AN: Small cell neuroendocrine carcinoma of the cervix: A human papillomavirus type 18-associated cancer. *Am J Surg Pathol* 1991;15:28-32.
210. Harris NL, Scully RE: Malignant lymphoma and granulocytic sarcoma of the uterus and vagina: A clinicopathologic analysis of 27 cases. *Cancer* 1984;53:2530-2545.
211. Gomez-Fernandez CR, Ganjei-Azar P, Capote-Dishaw J, Nadji M: Reporting normal endometrial cells in Pap smears: An outcome appraisal. *Gynecol Oncol* 1999;74:381-384.
212. Sarode VR, Rader AE, Rose PG, et al.: Significance of cytologically normal endometrial cells in cervical smears from postmenopausal women. *Acta Cytol* 2001;45:153-156.
213. Cherkis RC, Patten SF, Andrews TJ, et al.: Significance of normal endometrial cells detected by cervical cytology. *Obstet Gynecol* 1988;71:242-244.
214. Zucker PK, Kasdon EJ, Feldstein ML: The validity of Pap smear parameters as predictors of endometrial pathology in menopausal women. *Cancer* 1985;56:2256-2263.
215. Avis NE, McKinlay SM: The Massachusetts Women's Health Study: An epidemiologic investigation of the menopause. *J Am Med Womens Assoc* 1995;50(2):45-49.
216. Ng ABP, Regan JW, Hawliczek S, Wentz B: Significance of endometrial cells in the detection of endometrial carcinoma and its precursors. *Acta Cytol* 1974;18:356-361.
217. Montz FJ: Significance of "normal" endometrial cells in cervical cytology from asymptomatic postmenopausal women receiving hormone replacement therapy. *Gynecol Oncol* 2001;81:33-39.
218. Koss LG, Durfee GR: Cytologic diagnosis of endometrial carcinoma: Results of ten years of experience. *Acta Cytol* 1962;6:519-531.
219. Nguyen TN, Bourdeau JL, Ferenczy A, Franco EL: Clinical significance of histiocytes in the detection of endometrial adenocarcinoma and hyperplasia. *Diagn Cytopathol* 1998;19:89-93.
220. Tambouret R, Bell DA, Centeno BA: Significance of histiocytes in cervical smears from peri/postmenopausal women. *Diagn Cytopathol* 2001;24:271-275.
221. Thrall MJ, Kjeldahl KS, Savik K, et al.: Significance of benign endometrial cells in Papanicolaou tests from women aged > or = 40 years. *Cancer* 2005;105(4):207-216.
222. Kapali M, Agaram NP, Dabbs D, et al.: Routine endometrial sampling of asymptomatic premenopausal women shedding normal endometrial cells in Papanicolaou tests is not cost effective. *Cancer* 2007;111(1):26-33.
223. Bean SM, Connolly K, Roberson J, et al.: Incidence and clinical significance of morphologically benign-appearing endometrial cells in patients age 40 years or older: The impact of the 2001 Bethesda System. *Cancer* 2006;108(1):39-44.
224. Browne TJ, Genest DR, Cibas ES: The clinical significance of benign-appearing endometrial cells on a Papanicolaou test in women 40 years or older. *Am J Clin Pathol* 2005;124(6):834-837.
225. Opjorden SL, Caudill JL, Humphrey SK, Salomao DR: Small cells in cervical-vaginal smears of patients treated with tamoxifen. *Cancer* 2001;93(1):23-28.

Respiratory Tract

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METASTATIC CANCERS TO THE LUNG

Exfoliative cytology was first used to study cells of the respiratory tract in 1845.¹ The ability to diagnose pulmonary diseases cytologically was appreciated as early as 1919,² but it was not until the 1950s and 1960s that pulmonary cytology came into its own as a diagnostic discipline. Its emergence was bolstered by the introduction

of direct sampling methods via bronchoscopy and fine-needle aspiration (FNA),³ resulting in an armamentarium of sampling techniques. Since then, the improved sensitivity (and common application) of thoracic imaging has created an ever-increasing need for the cytologic evaluation of pulmonary lesions.

NORMAL ANATOMY, HISTOLOGY, AND CYTOLOGY OF THE RESPIRATORY TRACT

The respiratory tract can be categorized into upper and lower compartments. The upper airway extends from the sinonasal region to the larynx. The cells of the upper airway are occasionally seen in lower respiratory tract specimens. The lower respiratory tract, which is the major focus of diagnostic respiratory cytopathology, extends from the trachea to the lungs. The tracheobronchial tree divides into progressively smaller units: bronchi, bronchioles, and respiratory acini.

Anatomy and cellular components of the respiratory tract:

Upper respiratory tract:

- ciliated columnar cells
- squamous cells

Lower respiratory tract:

- trachea and bronchi
 - ciliated columnar cells
 - goblet cells
 - basal or reserve cells
 - neuroendocrine cells
- terminal bronchioles
 - nonciliated cuboidal or columnar cells (Clara cells)
- alveoli
 - type I and II pneumocytes
 - alveolar macrophages

The trachea and bronchi are lined by a pseudostratified epithelium. The predominant cell is the *ciliated columnar cell*, which has a basally placed nucleus and finely textured chromatin. The luminal surface has a thick terminal bar with cilia (Fig. 2.1). *Goblet cells*, present in a ratio of approximately one per six ciliated cells, also have basally located nuclei, but they lack cilia and their cytoplasm is distended by mucus. The goblet cells secrete mucus, and the ciliated cells move the mucus and entrapped contaminants up the airway. Adjacent to the basement membrane are *basal* or *reserve cells*: small, undifferentiated cells that are the presumed forerunners of the ciliated and goblet cells. *Neuroendocrine cells*, or Kulchitsky cells, are also present in the respiratory epithelium, but they are identified only with special stains or ultrastructural examination; they are argyrophil-positive and possess dense-core granules.

The terminal bronchioles are lined by nonciliated cuboidal to columnar cells called *Clara cells*, usually not recognized as such on cytologic preparations. The alveolar lining consists of *type I* and *type II pneumocytes*. Type I pneumocytes, which are more numerous, are paper



Figure 2.1 Normal ciliated bronchial cells (bronchial brushing). These columnar cells have oval nuclei and finely stippled chromatin. Numerous cilia project from the apical surface.

thin and cover the gas exchange portion of the alveolar surface. The type II pneumocyte is more conspicuous: plump and cuboidal rather than flat. It secretes pulmonary surfactant, seen ultrastructurally as osmiophilic lamellar bodies. After lung injury, these cells function as reserve cells for the delicate type I pneumocyte. On cytologic preparations, type II pneumocytes are round and have vacuolated cytoplasm; they can be difficult to distinguish from macrophages.

Alveolar macrophages vary in appearance depending on the amount and type of phagocytosed cytoplasmic material. In general, they have one or more round to oval nuclei and lacy or bubbly cytoplasm (Fig. 2.2). Numerous alveolar macrophages must be identified for a sputum sample to be judged adequate. Under normal

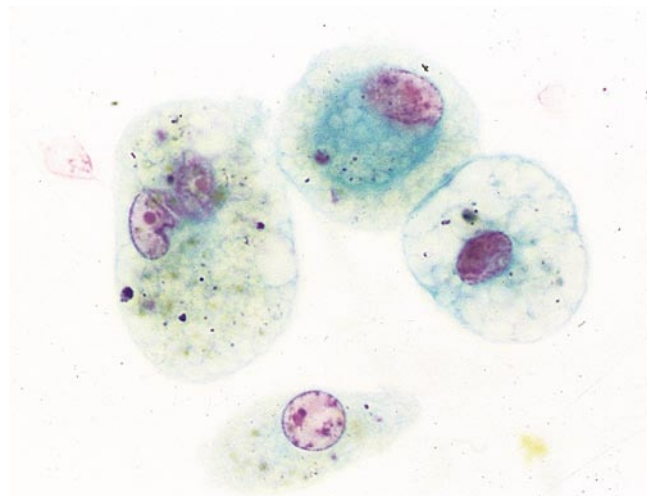


Figure 2.2 Pulmonary alveolar macrophages (sputum). Pulmonary macrophages have abundant foamy cytoplasm that often contains black carbon particles, as seen here. These macrophages fill with hemosiderin pigment following pulmonary hemorrhage. Hemosiderin is golden brown rather than black.

circumstances, a few white blood cells, such as neutrophils and lymphocytes, are also found within the alveolar compartment. An increased number of inflammatory cells is abnormal: Abundant neutrophils indicate an acute pneumonia, and numerous lymphocytes are usually associated with chronic inflammation.

SAMPLING TECHNIQUES, PREPARATION METHODS, REPORTING TERMINOLOGY, AND ACCURACY

Familiarity with the variety of sampling and preparation methods is crucial for cytologic interpretation because cytomorphology is different depending on the sampling and preparation method. The accuracy of respiratory cytology also varies depending on the specimen type.

As with other nongynecologic cytology specimens, respiratory tract diagnoses are typically reported as “negative for malignant cells,” “positive for malignant cells,” or “non-diagnostic (unsatisfactory),” followed by a descriptive diagnosis. Inconclusive diagnoses are commonly reported as “atypical cells present” (usually connoting a lower degree of suspicion) or “suspicious for malignancy (connoting a high degree of suspicion),” depending on the degree of suspicion. Cancer is confirmed in 40% of “atypical” respiratory specimens and in almost 70% of those diagnosed as “suspicious.”⁴ Atypical or suspicious cases remain inconclusive even after careful retrospective reexamination, which fails to reveal any morphologic features to reliably distinguish benign from malignant specimens.⁵

Sputum

Sputum consists of a mixture of cellular and noncellular elements that are cleared by the mucociliary apparatus. It was once the most common respiratory tract specimen because it is relatively easy to obtain and causes little if any discomfort. Unfortunately, screening asymptomatic smokers with sputum cytology does not decrease mortality from lung cancer. Today, sputum cytology is generally reserved for symptomatic individuals. Even here, with the advent of bronchoscopy and FNA, its use as the mainstay in respiratory cytology has declined significantly.

Collecting multiple sputum samples over several days optimizes sensitivity. Early morning, deep cough specimens are preferred.⁶ If the patient is not able to expectorate adequately, expectoration can be induced by having the patient inhale nebulized water or saline. Sputum induction increases the detection of lung cancer.⁷ When prompt preparation of sputum is not possible, the patient can expectorate into a 70% ethanol solution, which prefixes the specimen.

A simple method of sputum preparation is known as the “pick and smear” technique, whereby fresh sputum is examined for tissue fragments, blood, or both. Smears are prepared from areas that contain these elements and are immediately fixed in 95% ethanol. A modification of this is the Saccomanno method, which calls for sputum to be collected in 50% ethanol and 2% carbowax.⁸ The specimen is then homogenized in a blender and concentrated by centrifugation. Improved sensitivity has been demonstrated by the use of dithiothreitol (DTT) for homogenization.⁹ Smears are made from the concentrated cellular material. The Saccomanno method must be performed in a biologic safety hood as a result of the risks of infection from aerosolization. Sputum can also be processed using thinlayer methods or embedded in paraffin for cell block sections.¹⁰

The adequacy of a sputum sample is established by finding numerous pulmonary macrophages. They indicate that a deep cough specimen of the lower respiratory tract has been obtained. Specimens consisting merely of squamous cells, bacteria, and *Candida* organisms are unsatisfactory because they represent only oral contents. Even ciliated cells, which also line the sinonasal passages, do not guarantee that a sample is from the lower respiratory tract.

The sensitivity of sputum cytology for the diagnosis of malignancy increases with the number of specimens examined, from 42% with a single specimen to 91% with five specimens.¹¹ The specificity of sputum examination is high, ranging from 96% to 99%, and the positive and negative predictive values are 100% and 15%, respectively.¹² Negative sputum results do not guarantee the absence of a malignancy, especially in a patient suspected of having lung cancer. The sensitivity of sputum cytology depends on the location of the malignant tumor: 46% to 77% of central lung cancers but only 31% to 47% of peripheral cancers are diagnosed by sputum cytology.^{13,14} Surprisingly, sensitivity is independent of tumor stage and histologic type. Accuracy in tumor classification is 75% to 80%¹⁵ and is tumor type dependent.¹⁶

Bronchial Specimens

A pivotal improvement in sampling cells from the lower respiratory tract occurred with the development of the flexible bronchoscope in the late 1960s. Today, any part of the respiratory tract can be sampled with this device.

Complications of bronchoscopy are rare (0.5% and 0.8% for major and minor complications, respectively),¹⁷ and include laryngospasm, bronchospasm, disturbances of cardiac conduction, seizures, hypoxia, and sepsis. The incidence of major complications is higher for transbronchial biopsy (6.8%).¹⁷

Bronchial Aspirations and Washings

Bronchial secretions can be aspirated directly from the lower respiratory tract through the bronchoscope, but

an alternative (and more common) method is to “wash” the mucosa by instilling 3 to 10 mL of saline and aspirate the washings. The fluid is centrifuged and the concentrate used to make smears, thinlayer preparations, or cell blocks; the latter are particularly useful when special stains are needed.

Bronchial Brushings

Fiberoptic bronchoscopy allows direct visualization and sampling of the tracheobronchial tree. A brush is applied to the surface of an endobronchial lesion, and the entrapped cells are either smeared onto a glass slide or rinsed in a collection medium for thinlayer or cell block preparation. If smears are made, immediate fixation (by immersion into 95% ethanol or spray fixation) of the smears is essential to preserve morphologic detail.⁶

The diagnostic accuracy of bronchial washing or brushing cytology is comparable to that of bronchial biopsy.¹⁸ Brushings with cell block preparation sometimes detect malignancy more reliably than bronchial biopsies.¹⁹ Accuracy improves when clinical history is provided with the specimen.²⁰ The diagnostic yield also improves when several different sampling methods are used in concert.^{21,22}

Bronchoalveolar Lavage

The choice between using bronchoalveolar lavage (BAL) and bronchial washing depends on the location of the airway one desires to sample. With BAL, the bronchoscope is wedged into position as far as it will go, and distal airways are flushed with several aliquots of sterile saline. The first aliquot is more representative of the cellular material from larger airways, whereas subsequent aliquots reflect the alveolar compartment.

BAL is particularly useful for the diagnosis of opportunistic infections in patients who are immunocompromised. The specimen can be examined cytologically and a portion also submitted for microbiologic studies. The distinction between oral contamination and a real bacterial infection can be difficult, but an abundance of normal squamous cells usually indicates contamination by oral flora, whereas neutrophils imply a real infection.²³ In patients who are immunocompromised, the diagnostic yield for infectious pathogens is 39%, the sensitivity 82%, and the specificity 53%.²⁴ In patients with acquired immune deficiency syndrome (AIDS), BAL has a sensitivity for documenting infection that is comparable to that for transbronchial biopsy (86%); when used in combination with biopsy, the sensitivity increases to 98%.²⁵ Historically, the most common pulmonary pathogens that were detected by BAL in individuals who were human immunodeficiency virus (HIV) seropositive were *Pneumocystis carinii* (78%) and bacteria (19%); the remaining infections were the result of *Mycobacterium tuberculosis*, atypical mycobacteria, *Histoplasma*, and

Cryptococcus.²⁶ The frequency and distribution of infections has changed since the widespread use of highly active antiretroviral therapy (HAART) to treat HIV.²⁷ Among individuals who are HIV-seropositive with non-specific cytologic results, 27% prove to have pathogens, usually bacterial or fungal, by either culture or biopsy,²⁸ which emphasizes the importance of a multimodal approach to diagnosis in this setting.

BAL is also used for the diagnosis of malignancy, with sensitivity that ranges from 35% to 70%.^{29,30} The sensitivity of BAL for detecting malignancy is higher for multifocal or diffuse tumors like bronchioloalveolar carcinoma.³¹ False-positive results are occasionally encountered as a result of atypical type II pneumocytes in the setting of pneumonia, diffuse alveolar damage,³² bone marrow transplantation,³³ and chemotherapy.³⁴

Transbronchial Fine-Needle Aspiration

Transbronchial FNA is especially useful for the diagnosis of primary pulmonary lesions that lie beneath the bronchial surface and for staging lung cancer patients by sampling mediastinal lymph nodes.³⁵⁻³⁷ In these settings, the need for additional surgical procedures is eliminated in 20% of patients, and the cost is one third that of mediastinoscopy.³⁸ The lesion is aspirated with a retractable (Wang) needle passed through a flexible catheter that is sent down the bronchoscope.

When used to sample mediastinal lymph nodes, at least a moderate number of lymphocytes must be present to ensure the adequacy of the specimen and avoid a false-negative result.³⁹ Complications from transbronchial aspiration are rare and include endobronchial bleeding, which is usually controlled by suctioning. Contraindications are coagulopathy, respiratory failure, and uncontrollable coughing.⁴⁰

Transbronchial FNA augments the diagnostic accuracy of bronchial washings, brushings, and endoscopic biopsies for the detection of primary pulmonary neoplasms.^{41,42}

Accuracy of transbronchial fine-needle aspiration:

- sensitivity (FNA alone): 56%
- sensitivity (combined with bronchial brush, wash, and biopsy): 72%
- specificity: 74%
- positive predictive value: 100%
- negative predictive value: 53% to 70%

Transbronchial FNA is accurate in distinguishing small cell from non-small cell lung cancer.⁴³ For mediastinal staging of bronchogenic carcinoma, the negative predictive value of transbronchial FNA increases from

36% to 78% when negative specimens without sufficient lymphocytes are regarded as unsatisfactory for evaluation.³⁹ The most common cause of false-negatives is sampling error.⁴² The accuracy of mediastinal staging improves with the use of ultrasound guidance.^{35,36}

Transesophageal Fine-Needle Aspiration

Mediastinal lymph node sampling can be performed not only bronchoscopically, but also endoscopically by passing the needle through the esophagus.⁴⁴⁻⁴⁶ The addition of ultrasound guidance improves the accuracy of mediastinal lymph node sampling.⁴⁷ Like bronchoscopic FNA, endoscopic FNA realizes significant cost savings⁴⁶ and reduces the number of unnecessary thoracotomies.⁴⁵

Endoscopic transesophageal FNA has a diagnostic accuracy (70% to 80%)^{48,49} that approaches that of ultrasound-guided transbronchial FNA (84% to 96%).^{37,44,45,47} When used in combination with transbronchial FNA, the diagnostic yield for mediastinal staging is greatly improved, with an accuracy that approaches 100%.⁵⁰ As with transbronchial FNA, a moderate number of lymphocytes must be present to ensure the adequacy of the specimen and avoid a false-negative result.

Percutaneous Fine-Needle Aspiration

The ease, rapidity of diagnosis, and minimal morbidity of percutaneous FNA make it an attractive alternative to surgical biopsy in the evaluation of the patient with a pulmonary mass. Thus, FNA is of greatest benefit to patients for whom it spares a more invasive surgical procedure. Surgical intervention, in fact, can be avoided in up to 50% of patients with clinically suspected lung cancer.⁵¹ There are some contraindications, however.

Relative contraindications to percutaneous fine-needle aspiration:

- chronic obstructive pulmonary disease (COPD)
- emphysema
- uncontrollable coughing
- uncooperative patient
- bleeding diathesis (i.e., anticoagulant therapy)
- severe pulmonary hypertension
- arteriovenous malformation
- cardiac disease
- suspected echinococcal cyst⁵²

The most common complication of percutaneous FNA is a pneumothorax. A radiographically detectable pneumothorax occurs in 21% to 34% of patients;⁵³ only 10%, however, require intercostal drainage tubes.⁵³ The risk of a pneumothorax increases with the number of

passes through aerated lung and decreases if the path does not traverse aerated lung.⁵³ Transient hemoptysis occurs in 5% to 10% of patients. Other complications are rare, and include hemopericardium, hemothorax, air embolism, tumor seeding, and death.^{53,54}

Percutaneous FNA is usually performed by radiologists using computed tomography (CT) or ultrasound guidance. The needle gauge ranges from 18 to 22, and many different types of needle devices are available. Although many radiologists prefer 22-gauge Chiba needles, these require repuncture if more than one pass is needed. Another choice is the coaxial needle, with a large-bore outer needle serving as the guide for a small-bore inner needle. Once the outer needle is positioned, more than one aspiration can be performed using the inner needle.

It can be helpful in some cases if a cytotechnologist or cytopathologist attends the FNA procedure to assist with specimen handling and assess its adequacy on-site. After direct smears are prepared, the needle is rinsed in a balanced electrolyte solution, Saccomanno's fixative, 50% ethanol, or commercial preservative solution. The cellular suspension can be processed for microbiologic analysis, cytospins, thinlayer preparations, filters, cell blocks, cytogenetic analysis, flow cytometric analysis, or electron microscopy. Formalin-fixed cell blocks are indispensable for histochemical and immunohistochemical stains. A decision regarding the need, if any, for special studies can be made by the cytotechnologist or cytopathologist after examination of the direct smears.

Percutaneous FNA is a reliable and accurate way to diagnose many pulmonary neoplasms.

Accuracy of percutaneous fine-needle aspiration:

- sensitivity: 89%
- specificity: 96%
- positive predictive value: 98%
- negative predictive value: 70%
- false-positive rate: 0.85%
- false-negative rate: 8%

In a study of more than 13,000 FNA specimens from 436 institutions, the diagnostic sensitivity was 89% for the procedure itself and 99% for the pathologist's interpretation.⁵⁵ This difference indicates that most false-negative results are the result of sampling error. About 15% of false-positive diagnoses and 5% of false-negative diagnoses have a significant, permanent, or grave influence on patient outcome.⁵⁵ The reliability of a negative FNA result is a matter of controversy, given that negative predictive values range from 34% to 88%.⁵⁶⁻⁵⁹ For this reason, most investigators recommend a repeat aspiration or tissue biopsy when a specific benign diagnosis that accounts for the lesion cannot be made with certainty.

The small, cutting biopsy method is no more accurate than FNA.^{56,60-62}

With regard to the management of patients with primary lung cancer, the most important consideration is to discriminate between small cell and non-small cell carcinoma of the lung, which is possible in more than 95% of cases diagnosed by FNA.⁶³

It is important to recognize that a variety of benign cells can occasionally contaminate a percutaneous FNA. Such cells need to be recognized as contaminants and not misconstrued as lesional. In particular, mesothelial cells from the pleura are common and in some cases they can be numerous (Fig. 2.3). They resemble the cells of a well-differentiated adenocarcinoma, but are identified as benign mesothelial cells by their relative flatness, cohesion, and the characteristic slitlike “windows” that separate the mesothelial cells from each other.

Contaminants of percutaneous fine-needle aspiration:

- cutaneous squamous cells
- mesothelial cells
- skeletal muscle
- fibroconnective tissue
- hepatocytes (transdiaphragmatic needle path)

If the specimen consists only of one (or more) of these contaminants, it should be interpreted as insufficient for evaluation (nondiagnostic) rather than negative because there is no evidence that the lesion itself has been sampled.

Capillary Wedge Samples

Pulmonary capillary blood samples are an uncommon specimen type. They are obtained using a vascular catheter in the wedge position and are useful in diagnosing disorders restricted to the pulmonary microvasculature. These include lymphangitic spread of carcinoma, amniotic fluid embolism, and fat embolism.⁶⁴ Blood is collected in a heparanized tube to prevent clotting, and diagnostic cells are concentrated using a Ficoll-Hypaque gradient.⁶⁵ Megakaryocytes, normally present in the pulmonary capillaries, confirm the proper site and are useful to determine specimen adequacy.⁶⁶ Because these are large, they may be mistaken for tumor cells, but their characteristic multilobulated nuclei permit correct identification.

BENIGN CELLULAR CHANGE

Benign Squamous Cell Changes

Benign squamous cells from the oral cavity often contaminate sputum and bronchial cytology specimens. Inflammatory conditions of the mouth caused by trauma, candidiasis, or pemphigus can exfoliate mildly atypical squamous cells (ASC) with hyperkeratinization and nuclear degeneration, usually in small numbers. Such minimal changes should not be misinterpreted as squamous cell carcinoma (SCC). More marked (but still benign) squamous cell atypias occur adjacent to cavitary fungal infections and stomas, and with almost any injury to the lung (e.g., infarction, radiation, chemotherapy, sepsis, diffuse alveolar damage), and might result

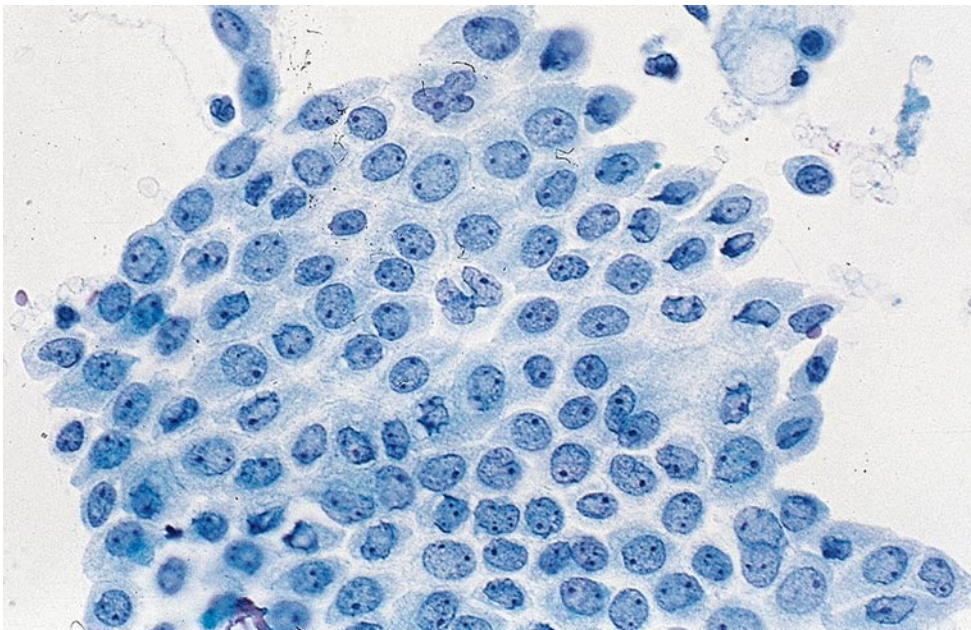


Figure 2.3 Mesothelial cells (fine-needle aspiration [FNA]). Benign mesothelial cells are occasionally seen in transthoracic fine-needle aspirates. They are arranged in flat, cohesive sheets. The cells have round or oval nuclei, small nucleoli, and a moderate amount of cytoplasm, and space between cells—windows—can be appreciated.

in a false-positive interpretation of SQC.^{33,34} Another, uncommon source of false-positives is malignant cells from head and neck cancers that contaminate sputum and bronchial specimens.⁶⁷

Benign Bronchial Cell Changes

Benign bronchial cell changes occur in response to noxious stimuli such as radiation, chemotherapy, and severe inflammation. Under such conditions, ciliated columnar cells can increase their nuclear area many times over, with multinucleation, coarsely textured chromatin, and large nucleoli (Fig. 2.4). Large clusters of bronchial cells known as “Creola bodies” (named after the first patient in whom they were recognized) are commonly seen in chronic airway diseases like asthma (Fig. 2.5). Goblet cells can also proliferate and exfoliate as large sheets or

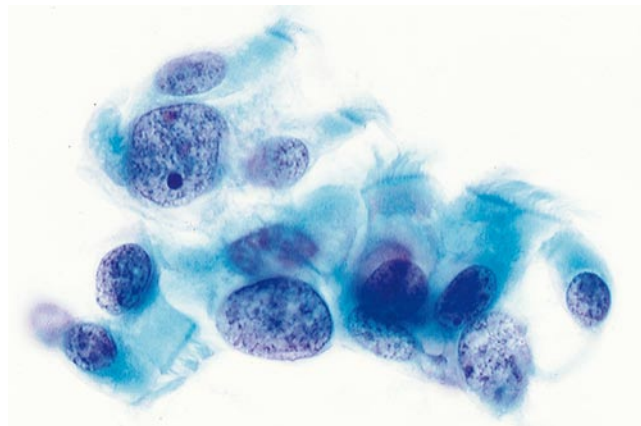


Figure 2.4 Reactive bronchial cells (bronchial brushing). Reactive bronchial cells may show marked nuclear size variation. Note that cilia—evidence of their benign nature—are retained.

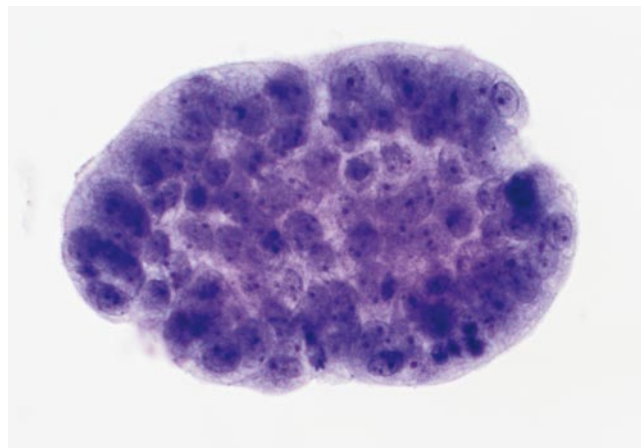


Figure 2.5 Reactive bronchial cells (Creola body; bronchial washing). In chronic lung diseases, as in this case of asthma, clusters of reactive bronchial cells may assume a spherical shape and resemble adenocarcinoma. Normal nuclear features and cilia indicate their benign nature. Eosinophils, a common finding in patients with asthma, are also present.

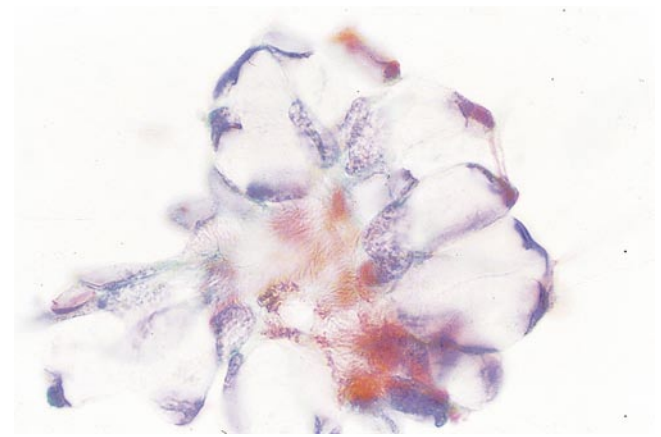


Figure 2.6 Goblet cell hyperplasia (bronchial brushing). In this group of benign bronchial cells, goblet cells, which have abundant mucin-filled cytoplasm, outnumber ciliated cells. The normal ratio of goblet cells to ciliated cells is altered in chronic diseases such as asthma and chronic bronchitis.

rounded clusters composed almost exclusively of goblet cells (Fig. 2.6). Goblet cell hyperplasia is a particularly notorious mimic of bronchioloalveolar carcinoma.

Marked benign bronchial cell changes (anisonucleosis, Creola bodies, goblet cell hyperplasia) mimic those of adenocarcinoma. Malignancy can be excluded if the atypical cells have cilia or demonstrate a spectrum of changes (from benign to markedly atypical) rather than the two distinct cell populations typical of a malignant sample obtained bronchoscopically. Note that in sputum and FNA specimens, a helpful dual cell population (malignant cells and bronchial cells) is usually not apparent.

Bronchial Reserve Cell Hyperplasia

As the surface epithelium of the respiratory tract is shed during lung injury, reserve cells proliferate and are seen in bronchial washings and brushings (Fig. 2.7).

CYTOMORPHOLOGY OF RESERVE CELL HYPERPLASIA:

- tightly packed cells
- small cells
- smudged dark chromatin
- nuclear molding
- scant cytoplasm
- no mitoses or necrosis

Reserve cell hyperplasia (RCH) is a mimic of small cell carcinoma but is usually easily distinguished from it. The cells of reserve cell hyperplasia show greater cohesiveness; they are smaller; have smudged chromatin; and there are no mitoses or necrosis.

Repair

Repair represents reepithelialization of an ulcer created by trauma, radiation, burns, pulmonary infarction,

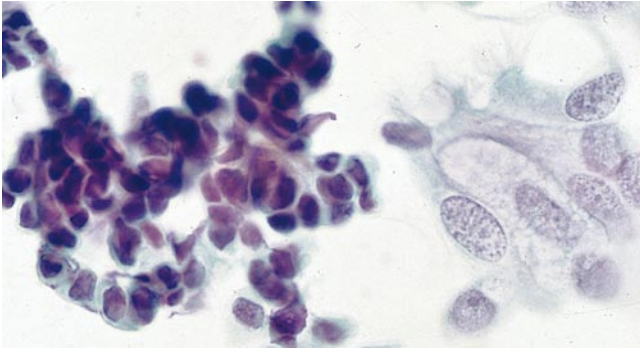


Figure 2.7 Reserve cell hyperplasia (bronchial brushing). These clusters of benign cells have hyperchromatic nuclei with nuclear molding. They are distinguished from small cell carcinoma (SMC) by their extremely small size and lack of necrosis. Compare these cells with the adjacent bronchial columnar cells.

and infections. Typical (and “atypical”) repair of the respiratory tract is similar to that seen in the cervix and gastrointestinal (GI) tract.

CYTOMORPHOLOGY OF REPAIR:

- flat, cohesive sheets
- abundant cytoplasm
- enlarged, often hypochromatic nuclei
- enlarged nucleoli
- mitoses

Reparative epithelium is most commonly seen in tracheobronchial brushings and washings. The differential diagnosis of repair includes malignancy: the non–small cell lung cancers, a metastasis, and other less common tumors. Malignant cells are usually less cohesive and orderly than reparative epithelium, and malignant cells are usually more numerous. Correlation with clinical history can be helpful; a conservative approach is recommended if the findings are not conclusive, and there is a history of mucosal trauma or other lung injury.

Type II Pneumocyte Hyperplasia

Because type II pneumocytes function as alveolar reserve cells, they proliferate after lung injury.

Causes of type II pneumocyte hyperplasia:

- pneumonia
- sepsis (diffuse alveolar damage)
- pulmonary embolus with infarction
- chemotherapeutic drugs
- radiation therapy
- inhalant damage (e.g., oxygen toxicity)
- interstitial lung disease



Figure 2.8 Type II pneumocyte hyperplasia (bronchoalveolar lavage [BAL]). In patients with acute lung injury, type II pneumocytes are markedly enlarged and may mimic adenocarcinoma, as seen here. This patient had diffuse infiltrates and marked respiratory distress resulting from diffuse alveolar damage. In this clinical setting, an unequivocal diagnosis of malignancy should be avoided, inasmuch as most patients with lung cancer are not acutely ill at presentation.

When floridly hyperplastic, as in diffuse alveolar damage, the cells of type II pneumocyte hyperplasia resemble those of adenocarcinoma (Fig. 2.8).

CYTOMORPHOLOGY OF TYPE II PNEUMOCYTE HYPERPLASIA:

- single cells and three-dimensional clusters
- large nuclei
- coarse chromatin
- prominent nucleoli
- scant to abundant cytoplasm

The only clue to avoiding an incorrect diagnosis of malignancy in a patient with type II pneumocyte hyperplasia may be the clinical history of respiratory distress and diffuse infiltrates. Thus, in a patient who is acutely ill with diffuse pulmonary infiltrates, markedly atypical cells should be interpreted cautiously.³³ Sequential respiratory specimens can be helpful, inasmuch as hyperplastic pneumocytes are not present in BAL specimens more than 1 month after the onset of acute lung injury.³²

NONCELLULAR ELEMENTS AND SPECIMEN CONTAMINANTS

Noncellular and extraneous elements in respiratory material can be inhaled, produced by the host, formed as a host response to foreign material, or introduced as laboratory contaminants.

Curschmann spirals are coiled strands of mucus. With the Papanicolaou stain, they appear as distinctive purple helices (Fig. 2.9). In the past they have been associated

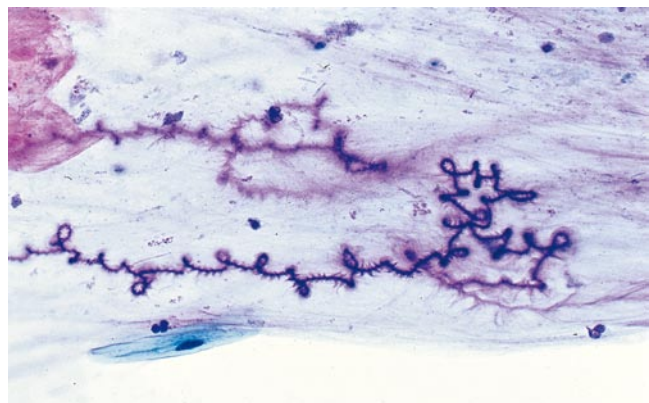


Figure 2.9 Curschmann spiral (sputum). These coils of inspissated mucus are commonly seen in respiratory specimens and are a nonspecific finding.

with chronic respiratory diseases, but they are, in fact, a nonspecific finding and not worth mentioning in the report. (Mentioning them might only cause puzzlement. Like some Hollywood celebrities, they are strikingly beautiful and equally irrelevant.)

Ferruginous bodies are mineral fibers encrusted with ferropoteins. Dumbbell-shaped, ranging from 5 to 200 μm in length, they stain golden-yellow to black with Papanicolaou stains. Some but not all ferruginous bodies contain a core of asbestos. So-called *asbestos bodies* are distinguished from other ferruginous bodies by their clubbed ends and thin, straight, lucent core. *Asbestos fibers* are not visible by light microscopy but are usually much more numerous than asbestos bodies. Patients with known asbestos exposure usually have high numbers of ferruginous bodies in BAL fluid.⁶⁸

Charcot-Leyden crystals are rhomboid-shaped, orangeophilic structures derived from degenerating eosinophils in patients with severe allergic disorders like asthma (Fig. 2.10).

Psammoma bodies are concentrically laminated calcifications seen in malignant tumors that have papillary

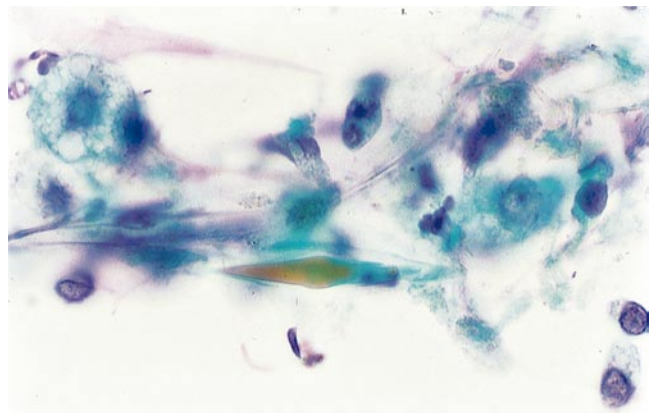


Figure 2.10 Charcot-Leyden crystal (bronchial washing). This needle-shaped crystal from a patient with asthma is a by-product of eosinophil degranulation.

architecture, like primary pulmonary adenocarcinoma, mesothelioma, metastatic thyroid or ovarian cancer. They are also seen in benign conditions like pulmonary tuberculosis and alveolar microlithiasis.

Corpora amylacea are spherical structures with circumferential and radiating lines. They measure between 30 and 200 μm and are indistinguishable from those seen in the prostate. They have no known significance but are more commonly seen in older individuals (Fig. 2.11).

Amorphous protein in respiratory specimens may be a clue to the diagnosis of amyloidosis or alveolar proteinosis.

Specimen contaminants include vegetable matter (Fig. 2.12), pollen, and the pigmented fungus *Alternaria* (Fig. 2.13A and B).

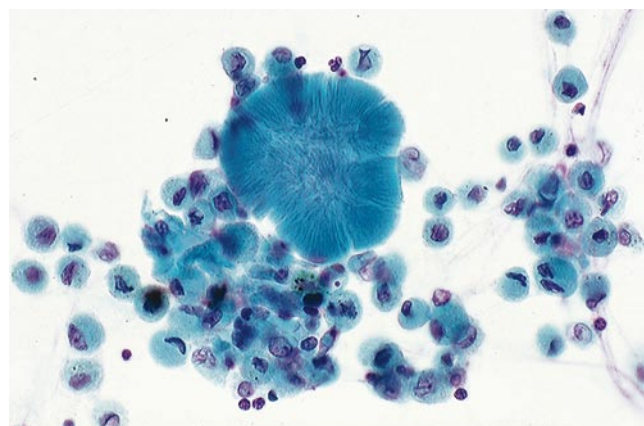


Figure 2.11 Corpora amylacea (bronchoalveolar lavage [BAL]). These large acellular bodies are somewhat variable in appearance. They may be spherical, as seen here, or angulated. They have fine radial striations and may have concentric laminations. Occasionally, there may be a central pigmented core. They are produced in the lung and other organs and have no known significance. Pulmonary corpora amylacea are not calcific, distinguishing them from psammoma bodies and the laminated spheres of pulmonary alveolar microlithiasis.

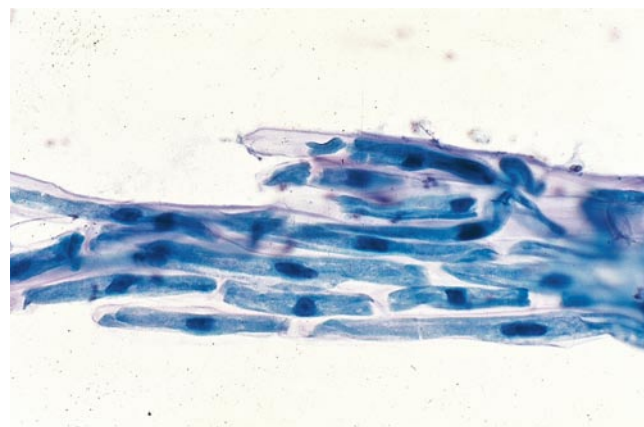


Figure 2.12 Vegetable cells (sputum). Some vegetable cells have elongated shapes and large nuclei, resembling the cells of keratinized squamous cell carcinoma (SQC). Their rectangular shape, uniform size, and refractile cellulose wall, however, help identify them as vegetable cells.

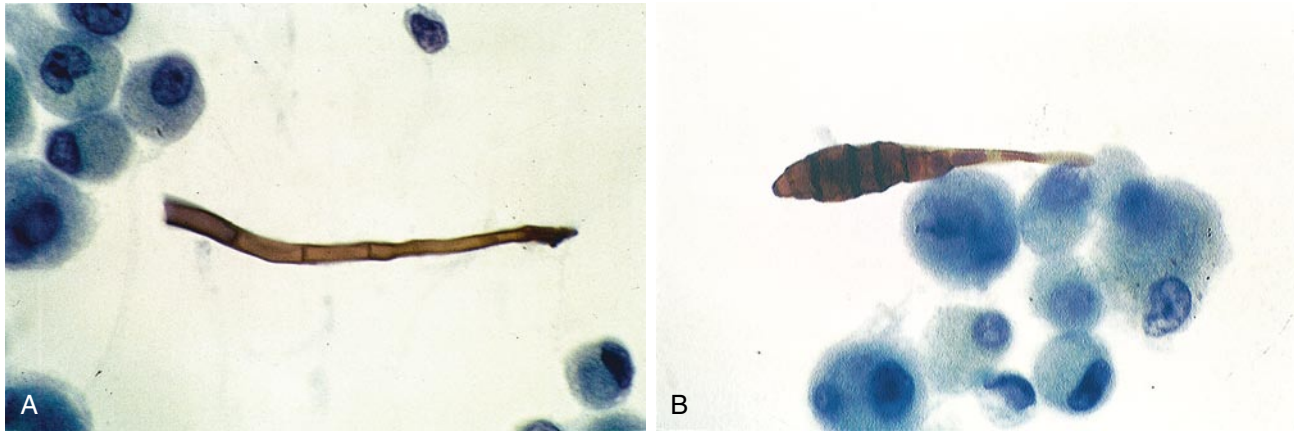


Figure 2.13 Alternaria (bronchoalveolar lavage [BAL]). This pigmented fungus is rarely pathogenic. It can contaminate virtually any cytologic specimen, including cervicovaginal smears, cerebrospinal fluid, and urine. A, The slender septate stalks (conidiophores) are occasionally branched (*not shown*). B, The conidia are snowshoe-shaped and have both transverse and longitudinal septations.

INFECTIONS

Cytology plays an important role in diagnosing infectious diseases, particularly those in patients who are immunocompromised, and is being used more frequently than ever because of improved sampling methods. It is important to know that conventional inflammatory responses can be much reduced, absent, or greatly altered in patients with immune deficiencies.

Viral Infections

Herpes Simplex

Herpes simplex virus (HSV) pharyngitis, laryngotracheitis, and pneumonia most commonly affect patients and neonates who are immunocompromised, and HSV-1 is the most common serotype to involve the respiratory tract. Ulcerative or necrotizing infections can involve the pharynx, larynx, tracheobronchial tree, or pulmonary parenchyma, and cytopathic changes are identical to those seen in other sites: multinucleation, nuclear molding, chromatin margination, and large nuclear (Cowdry A) inclusions ([Table 2.1](#)). The cytopathic changes of herpes simplex virus are identical to those of herpes zoster. If the cytomorphologic changes are equivocal, the diagnosis can be confirmed by viral culture, immunohistochemistry, or in situ hybridization.⁶⁹

Cytomegalovirus

Cytomegalovirus (CMV) is one of the most common of opportunistic infections. Patients with cytomegalovirus pneumonia often present with fever, dyspnea, cough, and diffuse nodular or reticular interstitial infiltrates. Viral cytopathic changes (cytomegaly, large basophilic nuclear and small basophilic cytoplasmic inclusions) are found in bronchial cells, pneumocytes, macrophages,

endothelial cells, and fibroblasts (see [Table 2.1](#)). The diagnosis can be confirmed by viral culture, immunohistochemistry, in situ hybridization, or the polymerase chain reaction.⁶⁹⁻⁷¹

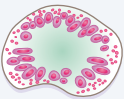
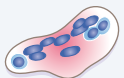
Measles Virus and Respiratory Syncytial Virus

Measles is a highly contagious, usually self-limited disease caused by the rubeola virus. The incidence has been curtailed as a result of the widespread use of a vaccine. However, measles pneumonia occurs as an opportunistic complication in children who are immunocompromised as a result of premature birth, cystic fibrosis, malignancy, or an immunologic disorder. Infection causes a giant cell pneumonia characterized by enormous multinucleated cells with cytoplasmic and nuclear inclusions (see [Table 2.1](#), [Fig. 2.14](#)).⁷² Similar findings are seen with infection by the respiratory syncytial virus (RSV). The diagnosis is usually confirmed by detecting respiratory syncytial virus antigen in BAL specimens.

Adenovirus

Adenovirus infection usually produces only a minor febrile illness, but adenovirus pneumonia can be severe and fatal, particularly in patients who are immunocompromised. The virus causes two types of nuclear inclusions. One is the smudge cell, in which a large basophilic inclusion usually fills the entire nucleus and obscures chromatin detail. The other is eosinophilic inclusions that resemble the Cowdry A inclusion of herpes simplex virus infection. A curious morphologic decapitation of the ciliated columnar cells, called ciliocytophthoria, can be prominent.⁷³ The detached cell apex, represented by only the terminal bar and cilia, without its nucleus, resembles a floating tuft of hair or eyelash (see [Table 2.1](#)).

TABLE 2.1 PULMONARY VIRAL INFECTIONS

Virus		Nuclear Features	Inclusions	Other Changes
Herpes simplex and herpes zoster		Multinucleation; molding; peripheral margination of chromatin	Eosinophilic, intranuclear (Cowdry type A)	—
Cytomegalovirus		Enlargement	Large intranuclear, basophilic, with halo; small cytoplasmic, basophilic	Cytoplasmic enlargement
Measles virus		Multinucleation	Eosinophilic, intranuclear; multiple eosinophilic intracytoplasmic	Giant cells
Respiratory syncytial virus		Multinucleation	Cytoplasmic, basophilic, with halo	Giant cells; necrosis
Adenovirus		Smudged appearance as a result of large inclusion that fills entire nucleus	Large intranuclear, basophilic	Ciliocytophthoria

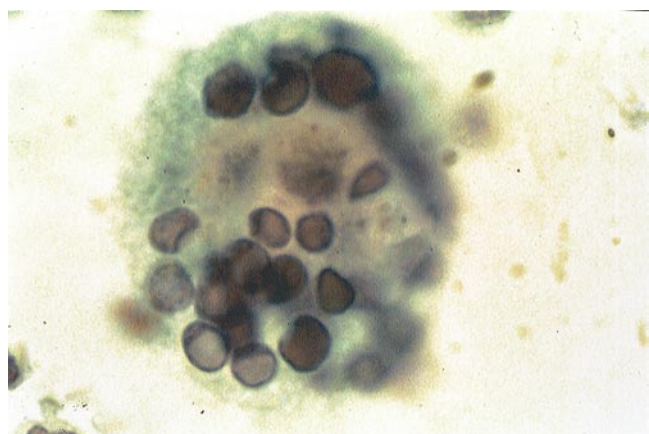


Figure 2.14 Measles virus cytopathic effect (bronchoalveolar lavage [BAL], cell block). Measles virus infection causes pneumonia with giant multinucleated epithelial cells that have eosinophilic intranuclear and intracytoplasmic inclusions. These cells are pathognomonic.

Bacterial Pneumonias

Bacterial pneumonias are caused by a large number of bacteria, but most are characterized by a neutrophilic exudate. Common organisms include *Streptococcus pneumoniae* (pneumococcus), other *Streptococci*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas* sp., *Legionella* sp., *Nocardia* sp., *Actinomyces* sp., and some anaerobic bacteria. Many but not all bacteria can be seen with routine stains and with the Gram stain. Cytologic examination is not usually employed for the diagnosis of a bacterial pneumonia, which is typically established by correlating clinical findings with microbiologic studies.

Bacterial pneumonias often have a characteristic lobar or lobular distribution, but some pneumonias appear as round and circumscribed masses on imaging studies

and thus mimic the appearance of a malignancy. In such cases, a cytologic specimen might be obtained, because the working clinical diagnosis is a suspected malignancy.

Several bacteria deserve special mention. *Actinomyces* species are a common inhabitant of the tonsillar area and thus a common contaminant of sputum and bronchial specimens (but not FNAs). Infection by *Actinomyces*, however, is surprisingly uncommon. Pulmonary infection occurs by aspiration of oral contents or by direct extension from subdiaphragmatic abscesses. Actinomycosis is usually a chronic infection that may result in sinus tracts. The bacteria aggregate into grossly visible sulfur granules (so-called because they look yellow on gross examination) and evoke a brisk neutrophilic response. When they appear in cytologic specimens as just an oral contaminant, *Actinomyces* bacteria are large blue “cotton balls” often associated with squamous cells, with no neutrophilic infiltrate, similar to what is occasionally encountered in Pap specimens (see Fig. 1.23). A true thoracopulmonary actinomycosis should be considered if the bacteria are associated with abundant neutrophils.

Nocardia are aerobic, filamentous bacteria that inhabit the soil and are acquired by inhalation. *N. asteroides* accounts for 80% of nocardial infections. Most patients are immunocompromised and have a subacute presentation. Cavitory nodules are seen in one third of patients. The organisms are found among abundant neutrophils. They are thin, filamentous, and beaded, with such extensive, predominantly right-angle branching that they resemble Chinese characters. They are gram-positive and stain with silver stains, but are only weak acid-fast and thus require modified acid-fast stains like the Fite-Faraco for visualization. The diagnosis is established by culture of a biopsy or BAL fluid.

Legionella pneumonia is caused by the aerobic gram-negative bacteria *Legionella* sp., of which the most common is *L. pneumophila*. The organisms are seen well with silver stains like the Steiner, Warthin-Starry, or Dieterle stains. A specific identification of *L. pneumophila* can be made in BAL samples using immunohistochemical or immunofluorescent methods.

Tuberculosis

Infection by *M. tuberculosis* commonly results in granulomatous inflammation (Fig. 2.15). Cytologic specimens contain aggregates of epithelioid histiocytes, lymphocytes, and Langhans giant cells. Necrosis may or may not be evident. Granulomas by themselves, however, are a nonspecific finding and can be seen in other conditions like fungal infections and sarcoidosis. A definitive diagnosis of tuberculosis rests on identifying the organisms with the help of a special stain (Ziehl-Neelsen) or by microbiologic culture. Cell block preparations are particularly useful for special stains, but rarely are more than one or just a few organisms identified. (By contrast, infection by *M. avium intracellulare*, as seen in patients who are immunocompromised patients, often yields innumerable acid fast organisms.) A sensitive (93%) and specific (99%) assay called the Mycobacterium Tuberculosis Direct Test (MTD) is also available for the detection of *M. tuberculosis* and can be applied to respiratory specimens like sputum.⁷⁴ The assay amplifies *M. tuberculosis* ribosomal ribonucleic acid (RNA) by the polymerase chain reaction.

In countries where *M. tuberculosis* is prevalent, the yield of acid-fast bacteria among all clinically suspicious lung masses can be quite high.^{75,76} In patients who are immunocompromised with tuberculosis, there may

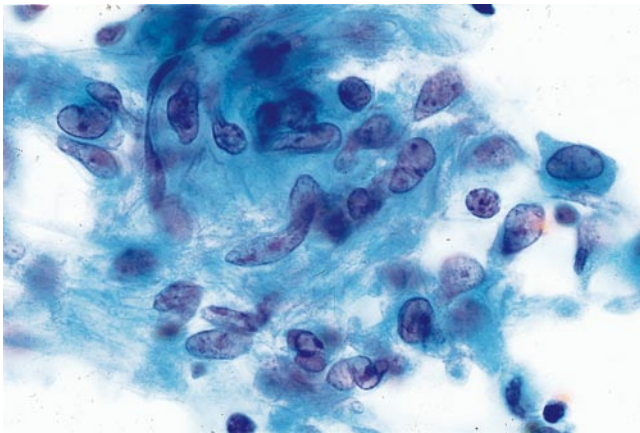


Figure 2.15 Granuloma (fine-needle aspiration [FNA], *M. tuberculosis* infection). The nodular aggregate of epithelioid histiocytes, which defines the granuloma, has a syncytial appearance because individual cell borders are indistinct. Note the curved and elongated, boomerang shape of some of the histiocyte nuclei. Interspersed lymphocytes can also be seen.

be an abundance of acid-fast organisms but few well-formed granulomas. If Romanowsky-type stains are used in such cases, the abundant acid-fast organisms can be identified as negative images.

Pulmonary Fungal Infections

Pulmonary fungal infections are readily diagnosed by cytology, particularly in transthoracic FNAs, and should be suspected whenever there is granulomatous inflammation or necrosis. Cell block material can be used for silver or periodic acid-Schiff (PAS) stains. Many fungi have a characteristic microscopic appearance that enables a rapid, specific diagnosis (Table 2.2).

Cryptococcosis

Cryptococcus neoformans is an inhabitant of the soil and is found in bird droppings. It can act as both a primary and an opportunistic pathogen and may involve numerous body sites. Pulmonary invasion by this fungus is heralded clinically by a productive cough, fever, and weight loss.

Histoplasmosis

Histoplasma capsulatum, another soil inhabitant, is contracted by the inhalation of spores and more commonly affects patients who are immunocompromised. The disease can mimic tuberculosis clinically, in that peripheral nodular lesions and mediastinal lymphadenopathy are relatively common. The organism, often present within the cytoplasm of macrophages, is so small that it may be overlooked if silver stains are not used.

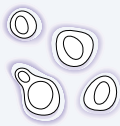
Blastomycosis

Blastomyces dermatitidis inhabits wooded terrain. Although the lung is the primary target of infection, there may be distant spread to other organs, such as skin, bone, and the urinary tract.

Coccidioidomycosis

Coccidioides immitis infection is quite common in endemic areas of the Southwest and Western United States, giving rise to positive skin tests in more than 80% of individuals in these areas. It produces a respiratory infection that usually resolves spontaneously, but persists as a pulmonary mass in about 2% of patients. Multiorgan dissemination is more common in the patient who is immunocompromised. Because BAL or bronchial washings detect less than 50% of culture-positive cases,⁷⁷ cytologic diagnosis is best documented by transthoracic FNA. The organisms appear as mature (sporulating) or immature spherules, often with a fractured (broken ping-pong ball) appearance, and free

TABLE 2.2 PULMONARY FUNGAL INFECTIONS

Disease	Organism	Demographics	Morphology	Size	Immunocompetent Response	Appearance
Cryptococcus	<i>Cryptococcus neoformans</i>	Worldwide	Yeast; variably sized; narrow-based budding; mucin capsule (immuno-competent); refractile center	4–15 μm (diameter)	Granulomatous	
Histoplasmosis	<i>Histoplasma capsulatum</i>	Americas, especially Ohio and Mississippi river valleys	Small budding yeast; intracellular	1–5 μm (diameter)	Granulomatous	
Blastomycosis	<i>Blastomyces dermatitidis</i>	North America	Broad-based budding yeast; thick cell wall	8–20 μm (diameter)	Neutrophilic/granulomatous	
Coccidioidomycosis	<i>Coccidioides immitis</i>	North American deserts	Spherules; endospores	15–60 μm ; 1–2 μm (diameter)	Granulomatous	
Paracoccidioidomycosis	<i>Paracoccidioides brasiliensis</i>	Central and South America	Yeast with multiple budding ("mariner's wheel")	4–40 μm (diameter)	Neutrophilic/granulomatous	
Sporotrichosis	<i>Sporothrix schenckii</i>	Worldwide	Intracellular ovoid yeast with slight halo	2–4 μm (diameter)	Neutrophilic/granulomatous	
Aspergillosis	<i>Aspergillus fumigatus</i> ; <i>A. flavus</i>	Worldwide	Hyphae septate; 45-degree angle branching; occasional fruiting bodies in cavities	Hyphae 10–30 μm (width)	Tissue/vascular invasion; colonization; fungus balls	
Phycomycosis (Zygomycosis)	<i>Mucor</i> ; <i>Absidia</i> ; <i>Rhizopus</i> ; <i>Cunninghamella</i>	Worldwide	Hyphae variably sized, ribbon-like nonseptate; 90-degree angle branching	Hyphae 10–30 μm (width)	Tissue/vascular invasion	
Candidiasis	<i>Candida albicans</i> ; <i>C. tropicalis</i> ; <i>C. glabrata</i>	Worldwide	Budding yeast forming pseudohyphae ("sausage links")	2–10 μm (width)	Respiratory space colonization; tissue invasion	

endospores (see Table 2.2). These are present on a background of granular eosinophilic debris with little inflammation. Hyphae are seen in 5% of cases. FNA cytology is diagnostically far superior to the culture of aspirated materials.⁷⁸

Paracoccidioidomycosis

Paracoccidioidomycosis, also known as South American blastomycosis, is caused by the dimorphic fungus *Paracoccidioides brasiliensis* (Fig. 2.16) and is the most common systemic mycosis in Latin America.⁷⁹ It frequently involves the lungs and mucocutaneous areas of healthy individuals, and clinically simulates tuberculosis. There may be a hyperplasia of the bronchial epithelium overlying granulomas, which may lead to the

erroneous diagnosis of SQC in cytologic material (see Fig. 2.25).⁸⁰ There is a high sensitivity (50% to 90%) for the detection of the organism in cell block preparations of sputum.⁸¹

Sporotrichosis

Pulmonary infection caused by *Sporothrix schenckii* is uncommon and occurs mainly in patients who are immunocompromised, including diabetics and alcoholics. The clinical symptoms are nonspecific. Though often self-limited, these infections can become chronic, with mass lesions or cavitary nodule formation. The yeasts resemble *Cryptococcus*, *Histoplasma*, and *Candida*, and therefore culture or fluorescent antibody staining is necessary for definitive diagnosis.⁸²

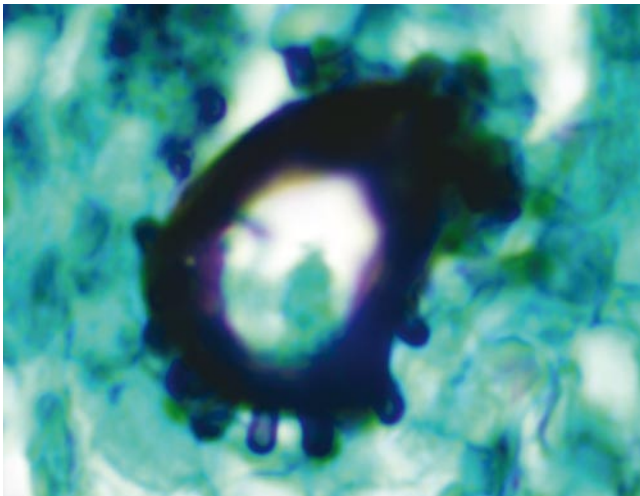


Figure 2.16 Paracoccidioidomycosis. This silver stain highlights the ship's wheel appearance of yeast forms budding off of the central parent yeast. The resemblance of the broad-based budding pattern to that of *Blastomyces* species is the reason for its alternative term, *South American Blastomycosis*.

Invasive Fungi

This subgroup of fungi characteristically invades pulmonary tissue, especially blood vessels, of patients who are immunocompromised. These organisms are readily diagnosed by transthoracic FNA.

Aspergillosis. *Aspergillus* species can cause a variety of pulmonary disorders. Bronchopulmonary aspergillosis is characterized by the expectoration of mucus plugs containing fungal organisms, numerous eosinophils, and Charcot-Leyden crystals. When invasive, there may be hemorrhagic necrosis caused by mycelial invasion of vessels. In cavitary lesions, the organisms sporulate, producing fruiting heads, and are associated with polarizable calcium oxalate crystals. There may be an associated squamous cell atypia (Fig. 2.25) that can be indistinguishable from carcinoma, making clinical correlation imperative.³⁴

Zygomycosis. Zygomycosis is caused by several mycelia-forming fungi, including *Mucor*, *Absidia*, *Cunninghamella*, and *Rhizopus*. They are angioinvasive and often cause infarctions in patients who are debilitated.

Candidiasis. *Candida* pneumonia is a common opportunistic infection. An elevated level of *Candida* antigen in BAL fluid suggests true infection rather than colonization.⁸³

Pneumocystis carinii

The taxonomy of this organism has been debated, but microbiologists favor classifying it as a fungus, based in part on molecular evidence.^{84,85} The pneumonia caused by *Pneumocystis carinii* is particularly common in individuals who are immunocompromised but has decreased in frequency in these patients since the advent of novel therapies.²⁷ The clinical presentation includes

dry cough, fever, and dyspnea. Pulmonary infiltrates are usually bilateral.

The organisms are well demonstrated in BAL material, which has a sensitivity that compares favorably with transbronchial biopsy.⁸⁶ They can also be detected in bronchial washings and induced sputum.⁸⁷ With Papanicolaou stains, the organisms themselves are not visible, but masses of organisms enmeshed in proteinaceous material result in green, foamy alveolar casts that are more circumscribed than debris or lysed red blood cells (Fig. 2.17A). The cysts are visualized with silver stains. They are cup-shaped, measure 5 to 7 μ m in diameter, and often have a central dark zone (Fig. 2.17B). No budding occurs, which helps distinguish them from *Histoplasma*. The Giemsa stain highlights the cysts as negative images, but the eight 1.5 μ m intracystic bodies or trophozoites are stained as discrete blue dots either within the cysts or as free organisms (Fig. 2.17C). In some cases, the foamy alveolar casts are absent, and the organisms may be present only in vacuolated macrophages.⁸⁸

Direct immunofluorescence has higher sensitivity (up to 92%) than the Giemsa, toluidine blue, and silver stains.^{89,90} Application of this method to induced sputum (Fig. 2.17D) is the preferred initial diagnostic step because it is noninvasive and highly accurate.

Strongyloidiasis

Pulmonary strongyloidiasis is caused by the nematode *Strongyloides stercoralis*. It can affect persons who are immunocompetent but is more common in patients who are immunosuppressed and presents as a pneumonitis with hemoptysis. Infection of the lungs is caused by the hematogenous migration of the infective larva (filariform larva) from the gut or skin. Histologically, there is a hemorrhagic pneumonia. This organism is identified in sputum or bronchial material by its large size and is differentiated from other hookworms by its notched tail and short buccal cavity (Fig. 2.18).

Dirofilariasis

Dog heartworm (*Dirofilaria immitis*) infection of the human lung has been documented by FNA.⁹¹ This microfilarial disease is transmitted from dogs to humans via mosquitos, resulting in entrapment within small pulmonary vessels and subsequent pulmonary infarction. Diagnosis is made by aspiration of the discrete peripheral nodule, which shows necrotic material, fragments of infarcted pulmonary tissue, chronic inflammation, a granulomatous response, and rarely the worm itself.

Echinococcosis (Hydatid Disease)

The clinical and cytologic features of Echinococcosis are described in Chapter 12 (see Fig. 12.4). Sputum

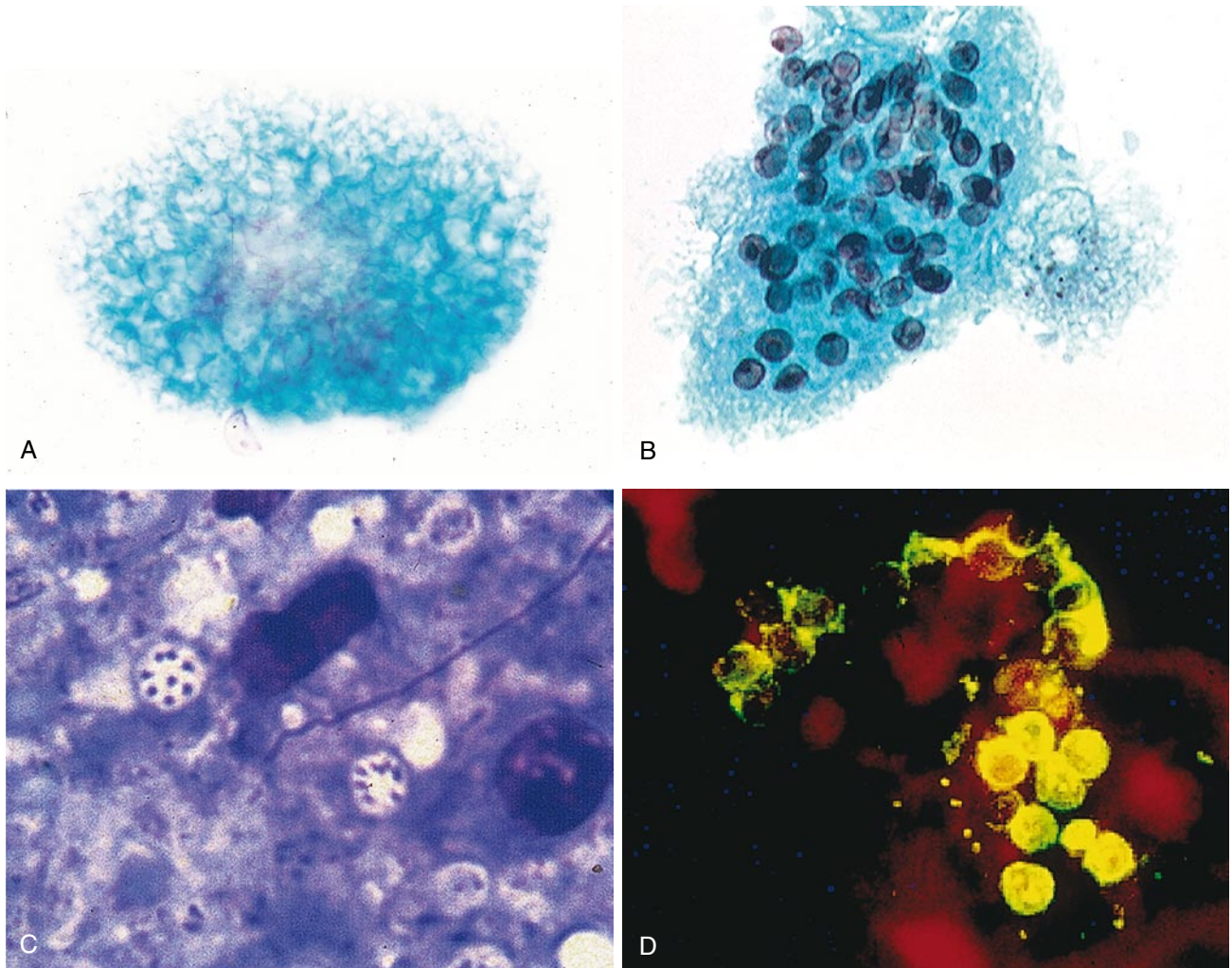


Figure 2.17 *Pneumocystis carinii* (bronchoalveolar lavage [BAL]). *A*, With the Papanicolaou stain, the pneumocystis organisms are not seen, but foamy proteinaceous spheres characteristic of this infection are identified. *B*, The cysts, which have a cup-shaped configuration and a central dark zone, are seen with the methenamine silver stain. *C*, The Giemsa stain outlines the cysts as negative images, and stains the intracystic bodies or trophozoites. Each cyst, as seen here, contains eight intracystic bodies. *D*, The direct immunofluorescence test is highly sensitive, revealing green fluorescent-stained organisms and their extracellular products.

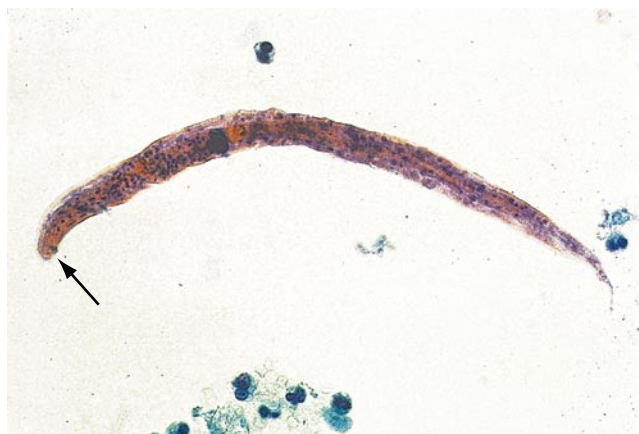


Figure 2.18 *Strongyloides* (sputum). These large round-worms are distinguished by their short buccal cavities (arrow).

may contain scoleces if pulmonary hydatid cysts rupture. Because of the risk of anaphylactic shock, aspiration of a clinically suspected hydatid cyst may be hazardous.^{52,92}

NON-NEOPLASTIC, NONINFECTIOUS PULMONARY DISEASES

Sarcoidosis

This common disease of unknown etiology is characterized by noncaseating granulomas in many organs, most commonly the lung.

CYTOMORPHOLOGY OF SARCOIDOSIS:

- aggregates of epithelioid histiocytes
- multinucleated giant cells
- lymphocytes

Cytologic specimens show noncaseating granulomas comprised of epithelioid histiocytes, with scattered lymphocytes and multinucleated, giant histiocytes (see Fig. 2.15).⁹³ The epithelioid histiocytes have round, oval, curved (boomerang-shaped), or spindle-shaped nuclei, with translucent, vacuolated cytoplasm. The epithelioid histiocytes are haphazardly arranged in a pseudosyncytial pattern.

Wegener Granulomatosis

This necrotizing vasculitis may present clinically as a lung mass with or without involvement of other organs like the nasal passages and kidneys. The histologic diagnosis of Wegener granulomatosis (WG) rests on the identification of necrosis, granulomatous inflammation, and vasculitis.

CYTOMORPHOLOGY OF WEGENER GRANULOMATOSIS:

- neutrophils
- giant cells
- necrotic collagen (“pathergic necrosis”)
- epithelioid histiocytes

The cytomorphic findings are nonspecific but include chunks of necrotic tissue (Fig. 2.19), giant cells, granulomas, and neutrophils. The differential diagnosis includes a necrotizing infection (e.g., tuberculous, fungal), lymphomatoid granulomatosis, and other uncommon pulmonary diseases. Thus, if suspected on the basis of characteristic cytomorphology, it can be

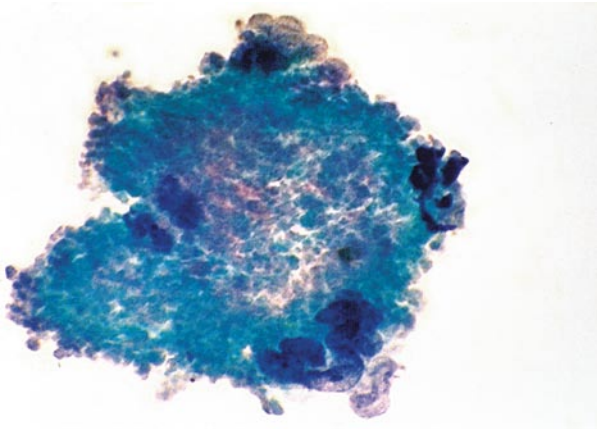


Figure 2.19 Wegener granulomatosis (fine-needle aspiration [FNA]). A bend-like, granular background debris consisting of necrotic collagen without acute inflammation is characteristic.

helpful to substantiate the diagnosis with serologic studies. The serum immunofluorescent antineutrophil cytoplasmic antibody (ANCA) test greatly aids in establishing the diagnosis of Wegener granulomatosis. Although the classic (cytoplasmic) pattern (c-ANCA) is considered more specific, neither the c-ANCA nor the perinuclear (p-ANCA) pattern is entirely sensitive or specific for the diagnosis of Wegener granulomatosis.⁹⁴

Pulmonary Amyloidosis

Pulmonary amyloidosis can be limited to the lung or represent a localized manifestation of systemic disease. Although it can affect a wide age range, most patients are in their 50s and 60s. Pulmonary amyloidosis can manifest itself as nodular parenchymal amyloidosis (a mass lesion whose imaging characteristics mimic those of a neoplasm or granulomatous disease, hence “amyloid tumor”); tracheobronchial amyloidosis (in which the deposits are mostly submucosal and result in dyspnea, wheezing, and recurrent pneumonia); or diffuse parenchymal amyloidosis (with diffuse or multifocal deposits).

FNA yields irregular, waxy, amorphous, hypocellular material with a scalloped, occasionally cracked appearance (see Fig. 9.11). These deposits appear blue-green on Papanicolaou stains and show apple-green dichroism under polarized light after staining with the Congo red stain. In the nodular parenchymal type there may be multinucleated giant cells, and calcification and ossification are common.

Pulmonary Alveolar Proteinosis

Pulmonary alveolar proteinosis (PAP) is a rare disease characterized by an accumulation of a lipid-rich material within alveoli. Pulmonary alveolar proteinosis is most likely the result of impaired macrophage function as a result of the production of neutralizing auto-antibodies to granulocyte-macrophage-colony-stimulating factor (GM-CSF).^{95,96} Pulmonary alveolar proteinosis can also be secondary to a number of conditions like HIV infection or lung transplantation.⁹⁴

The onset is insidious, and one third of patients are asymptomatic despite impressive radiologic abnormalities. There can be a nonproductive cough, dyspnea, and expectoration of gelatinous material. Examination of BAL specimens can be helpful. The characteristic findings include an opaque, milky gross appearance; large, acellular, eosinophilic, blobs that are positive for periodic acid-Schiff (Fig. 2.20); and pulmonary macrophages filled with material that is positive for periodic acid-Schiff. The differential diagnosis includes pulmonary edema, *P. carinii* pneumonia, and alveolar mucinosis. The diagnosis is made by correlating clinical findings with laboratory results and characteristic cytologic findings. Symptomatic patients are treated with whole lung lavage.

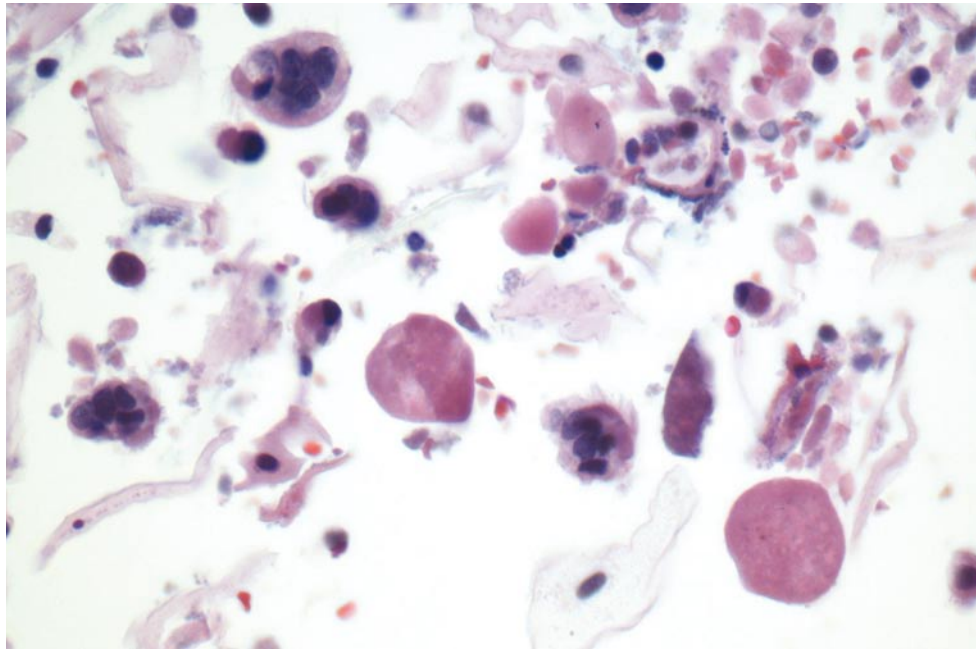


Figure 2.20 Pulmonary alveolar proteinosis (bronchoalveolar lavage [BAL]). Hematoxylin-eosin (H & E) stained cell block sections show numerous acellular eosinophilic aggregates.

Common Inflammatory Processes

There is considerable overlap in the cytologic features of common pulmonary diseases like organizing pneumonia (Fig. 2.21A and B), bronchiolitis obliterans obstructing pneumonia, diffuse alveolar damage, and transplant rejection. All show variable numbers of inflammatory cells such as macrophages, neutrophils, eosinophils, and lymphocytes.⁹⁷⁻¹⁰¹ Reactive pneumocytes are especially prominent in organizing pneumonia and diffuse alveolar damage (see Fig. 2.8).

As pneumonias organize, they can mimic mass lesions. Occasionally, the granulation tissue in these lesions may resemble Masson bodies (see Fig. 2.21B).

In the setting of lung transplantation, BAL is a routine monitoring procedure, especially for detecting infections, the most common of which are *Candida*, cytomegalovirus, and herpes simplex.¹⁰² Neutrophils are normally seen within the first 3 months of transplantation,¹⁰¹ and increased numbers of macrophages for years after the transplant.

BENIGN NEOPLASMS OF THE LUNG

Pulmonary Hamartoma

Despite their time-honored name, pulmonary hamartomas are, in fact, neoplasms. Recurrent clonal rearrangements have been identified involving the HMG1(Y) gene on chromosome 6p21.^{103,104} Most pulmonary

hamartomas are discovered incidentally as a solitary discrete, round mass in the lung periphery on thoracic imaging studies. Less commonly, they are central and endobronchial; multiple hamartomas are rare.

CYTOMORPHOLOGY OF PULMONARY HAMARTOMA:

- benign glandular cells
- immature fibromyxoid matrix and bland spindle cells
- mature cartilage with chondrocytes in lacunae
- adipocytes

FNA specimens show a mixture of mesenchymal (mainly fibromyxoid and cartilaginous material) and epithelial elements (Figs. 2.22A and B). In some hands, FNA has a sensitivity of 78% and a specificity of 100% in the diagnosis of a hamartoma.¹⁰⁵ A nationwide self-assessment testing program, however, revealed a general lack of familiarity with this lesion, with a troubling false-positive rate (22%). The most common misdiagnoses were carcinoma tumor, adenocarcinoma, and small cell carcinoma.¹⁰⁶ Hamartoma should be considered for any well-circumscribed neoplasm that contains epithelial cells.

Inflammatory Myofibroblastic Tumor

Inflammatory myofibroblastic tumor (IMT), once known as inflammatory pseudotumor, is a neoplasm of cytologically bland spindle cells that occurs in the lungs and at other sites. It is most common under the

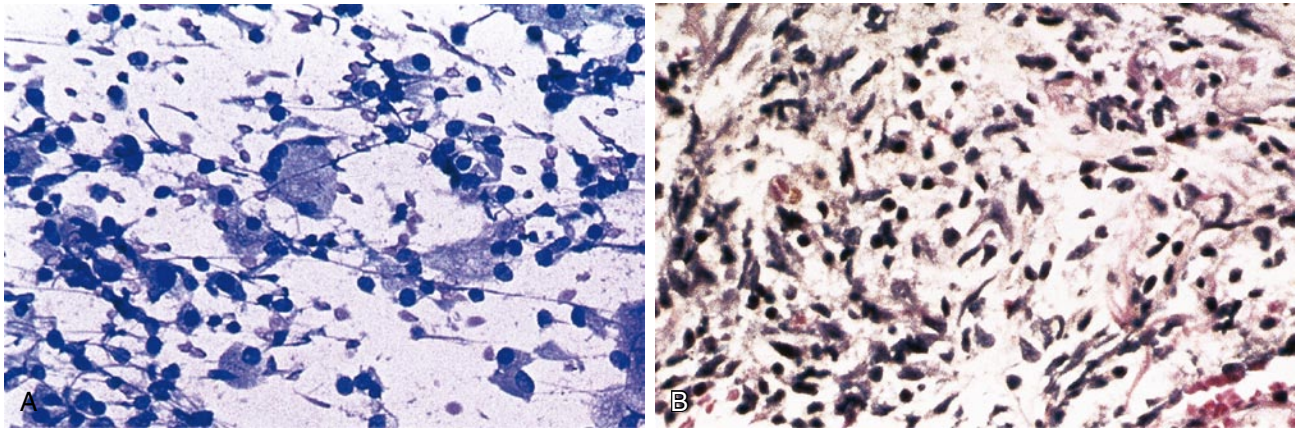


Figure 2.21 Organizing pneumonia (fine-needle aspiration [FNA]). Some pneumonias respond slowly to antibiotic treatment; such lesions may mimic a neoplasm radiographically. A, Smears typically show an admixture of lymphocytes and foamy macrophages. B, Cell block preparations show fragments of granulation tissue composed of loosely arranged fibroblasts admixed with chronic inflammatory cells.

age of 40. IMT is usually a peripheral, discrete, solitary nodule. These tumors behave unpredictably: most patients have an excellent prognosis, but some tumors are locally aggressive.¹⁰⁷ The neoplastic nature of this lesion was confirmed by the cloning of translocations involving the anaplastic lymphoma kinase (ALK) gene (chromosome 2p23) and the two tropomyosin genes, TPM3 and TPM4.¹⁰⁸ Anaplastic lymphoma kinase fusion partner variants include the clathrin heavy chain gene¹⁰⁹ and ATIC gene.¹¹⁰

CYTOMORPHOLOGY OF INFLAMMATORY MYOFIBROBLASTIC TUMOR:

- spindle cells
- storiform pattern
- polymorphous inflammatory cells
- minimal if any necrosis

Cytologic preparations show bland spindle cells arranged as fascicles or in a storiform pattern.¹¹¹ There is little pleomorphism and few mitoses. Admixed with the spindle cells is an impressive infiltrate of inflammatory cells, including lymphocytes, plasma cells, histiocytes, and Touton-type giant cells (defined as having a peripheral ring of nuclei). The differential diagnosis includes an organizing pneumonia and sarcomatoid carcinoma.

Endobronchial Granular Cell Tumor

Endobronchial granular cell tumors account for a small proportion of all granular cell tumors, which are thought to originate from Schwann cells. They are usually covered by bronchial epithelium and are composed of clusters of tumor cells with small nuclei and abundant, granular,

eosinophilic cytoplasm and are surrounded by a thickened basement membrane.

CYTOMORPHOLOGY OF ENDOBRONCHIAL GRANULAR CELL TUMOR:

- small clusters of macrophage-like cells
- abundant granular cytoplasm
- small, uniform, round to oval nuclei

On cytologic preparations, the cells are easily overlooked because of their similarity to macrophages (see Fig. 16.33).^{112,113}

PRENEOPLASTIC CHANGES OF THE RESPIRATORY EPITHELIUM

SQC of the lung is preceded by a precursor lesion akin to that of cervical cancer, with a progression from benign squamous metaplasia through dysplasia to invasive cancer. Unlike cervical cancer, however, lung cancer is only rarely associated with human papillomavirus (HPV) infection,¹¹⁴ and there is no successful method to screen for and treat precursor pulmonary lesions. Aberrant expression of specific cancer-associated proteins like p53 and the epidermal growth factor receptor (EGFR) tyrosine kinase in dysplastic respiratory epithelium precedes the development of invasive carcinoma.^{115,116} Although the precursor lesions are not as well defined as they are for the uterine cervix, most authors acknowledge degrees (mild, moderate, severe) of dysplasia. While, the classification of dysplasia is subjective,¹¹⁷ the risk of developing bronchogenic carcinoma increases with the degree of atypia, which is paralleled by the development of aneuploidy.¹¹⁸ More than 40% of patients with dysplasia are later diagnosed

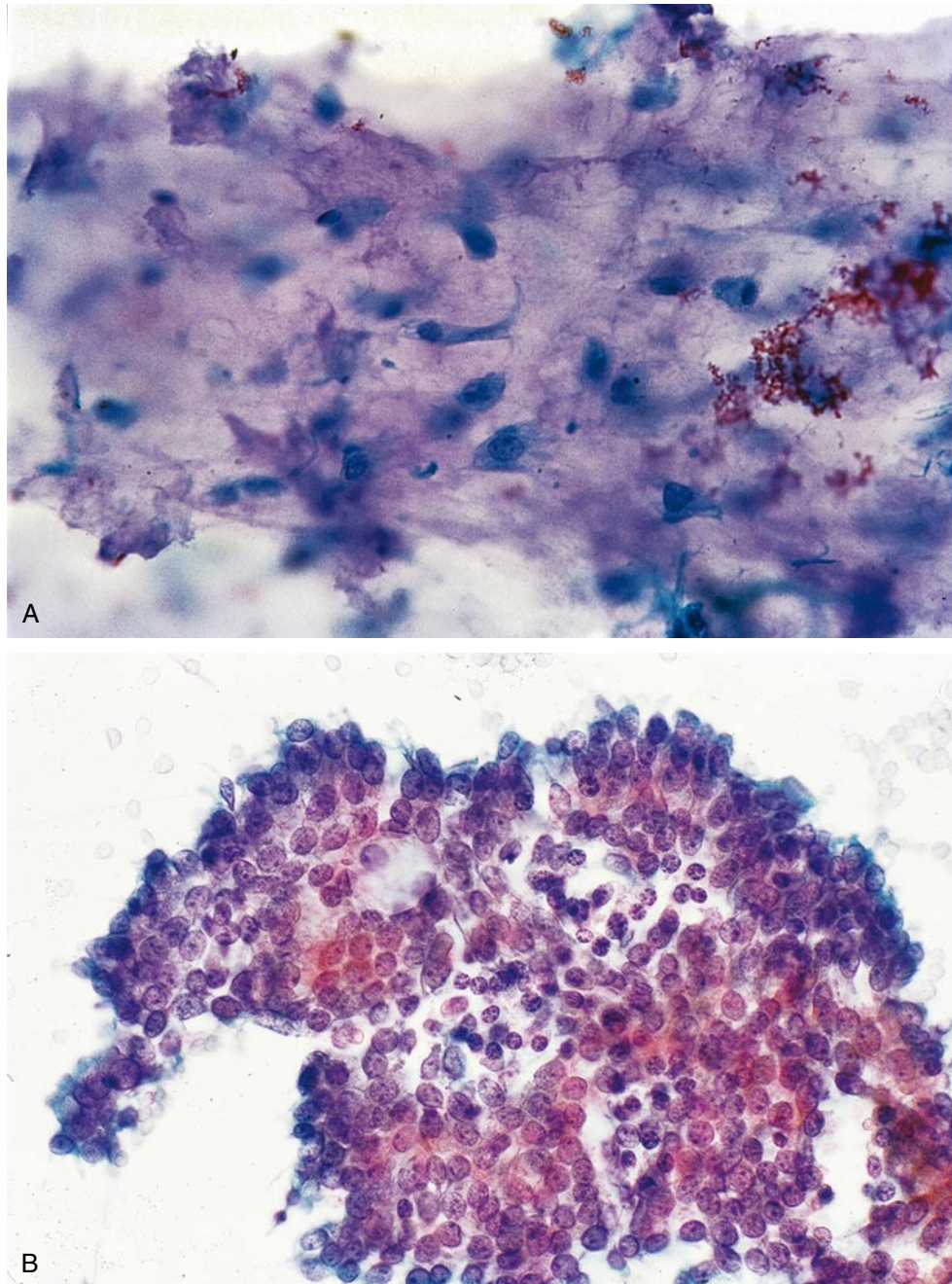


Figure 2.22 Pulmonary hamartoma (fine-needle aspiration [FNA]). A, Smears from a pulmonary hamartoma show fragments of myxoid material, chondroid material, or both. Chondrocytes in lacunae, which are green with Papanicolaou's stain, but unstained on hematoxylin-eosin (H & E) sections, are seen here. B, Epithelial cells and adipocytes (latter not shown) are also often present.

with SQC.¹¹⁹ Dysplastic changes have been demonstrated to regress in experimental systems.¹²⁰

LUNG CANCER

Carcinoma of the lung is the most common cancer in the world today. Its geographic distribution parallels expo-

sure to tobacco smoking, and, as a result, lung cancer is more common in developed countries. It is the most common fatal malignancy in both men and women in the United States, where it accounts for 31% of all cancer deaths in men and 26% in women.¹²¹

The causes of lung cancer are many. In men, 85% of lung cancers are attributed to cigarette smoking.¹²² Tobacco smoke contains numerous carcinogens, tumor

initiators, and radioactive compounds, but the biologic mechanism by which tobacco substances initiate lung cancer is not known. Genetic alterations play an important role. Chromosome 3p deletion (seen commonly in neuroendocrine tumors)¹¹⁸ and p53, K-ras, and p16 mutations are common in lung carcinomas.^{123,124} p53 mutation, the most frequent mutation in human cancers, likely occurs in lung cancer via a G-C to T-A transversion induced by cigarette smoke.¹²⁵ Exposure to asbestos has a synergistic effect with smoking: Asbestos workers who smoke increase their risk of developing pulmonary cancer by at least 50-fold. The development of lung cancer has been linked to exposure to other substances, including radon, crystalline silica, nickel compounds, and organic compounds like benzene and vinyl chloride.

Universal screening for lung cancer with chest radiographs and sputum cytology is not cost effective. The most comprehensive trial in the United States was undertaken by three medical centers under the auspices of the National Cancer Institute. Approximately 30,000 men were screened, and the overall mortality of the screened group was no lower than that of the control group.¹²⁶ High-risk individuals might benefit from periodic screening, however, like workers in the asbestos or uranium industries, persons over the age of 65 with a 20-year history of smoking, and those whose radiographs show a persistent abnormality.¹²⁷ The use of low-dose spiral computed tomography with sputum cytology has shown mixed results;¹²⁸⁻¹³² early cancer detection comes at the cost of unnecessary benign nodule detection and removal, and exposure to potentially harmful, repeated radiation exposures.^{128,133} Large, prospective, multi-institutional trials with high-risk patients are needed to prove the efficacy of low-dose spiral computed tomography with sputum screening.^{134,135} Detection of epigenetic or genetic aberrations in frequently mutated lung cancer genes (p53, K-ras, p16, HVAL2, FHIT, SFTPC) in sputum samples shows promise but is not yet widely applied.^{124,136,137}

Patients with lung cancer often present with shortness of breath, cough, chest pain, hoarseness, hemoptysis, or pneumonia. Some tumors, particularly adenocarcinomas, are detected incidentally in individuals who are asymptomatic.

Four histologic types account for 95% of all pulmonary tumors: SQC, adenocarcinoma, large cell carcinoma (LCC), and small cell carcinoma. Because it is usually metastatic at the time of detection, small cell carcinoma is treated with systemic chemotherapy. The treatment for the non-small cell carcinomas (SQC, adenocarcinoma, and LCC) is essentially identical: lobectomy or pneumonectomy for localized tumors. The subclassification of the non-small cell carcinomas is thus less important than the distinction between non-small cell and small cell carcinoma. Finer distinctions (histologic and even molecular) are becoming

more important as novel therapies are developed for specific lung cancer subtypes, like adenocarcinomas with EGFR mutations.

Lung cancers often show histologic heterogeneity. Almost 50% of lung cancers contain more than one histologic type.¹²² Thus, careful examination of all cytologic material for more than one histologic component is essential for correct classification.

Squamous Cell Carcinoma

SQC of the lung accounts for approximately one third of all primary pulmonary malignancies. Two thirds of these tumors occur in a central location, and the remainder arise in smaller bronchi. Postobstructive pneumonia and cavitation of the tumor are common. Of all the histologic subtypes, SQC is the one most commonly associated with hemoptysis. Apical SQCs in the superior sulcus are called Pancoast tumors, characterized by posterior rib destruction and neural invasion, resulting in severe pain and Horner syndrome (enophthalmos, ptosis, miosis, and ipsilateral decreased sweating). Several histologic variants (papillary, clear cell, small cell, basoid) have been described.¹²²

CYTOMORPHOLOGY OF WELL-DIFFERENTIATED SQUAMOUS CELL CARCINOMA:

- abundant dyshesive cells
- polymorphic cell shapes: polygonal, rounded, elongated (fiber-like), tadpole shaped
- dense cytoplasmic orangeophilia (Papanicolaou stain)
- pyknotic nuclei
- frequent anucleate cells

The cytologic features vary depending on the degree of squamous differentiation. In a well-differentiated SQC, the malignant cells are dyshesive, come in a variety of shapes (polygonal, spindle, tadpole, [Fig. 2.23A and B](#)), and have abundant smooth and dense cytoplasm that is filled with keratin. The cytoplasm stains green, yellow, or orange with the Papanicolaou stain and robin's egg blue with Romanowsky stains. Nuclei are usually small, hyperchromatic, and smudgy, and nucleoli are often inconspicuous.

CYTOMORPHOLOGY OF MODERATELY AND POORLY DIFFERENTIATED SQUAMOUS CELL CARCINOMAS:

- large, cohesive clusters of elongated cells
- rare to absent keratinization
- large nuclei
- coarse chromatin texture ("Idaho potato")
- ± prominent nucleoli

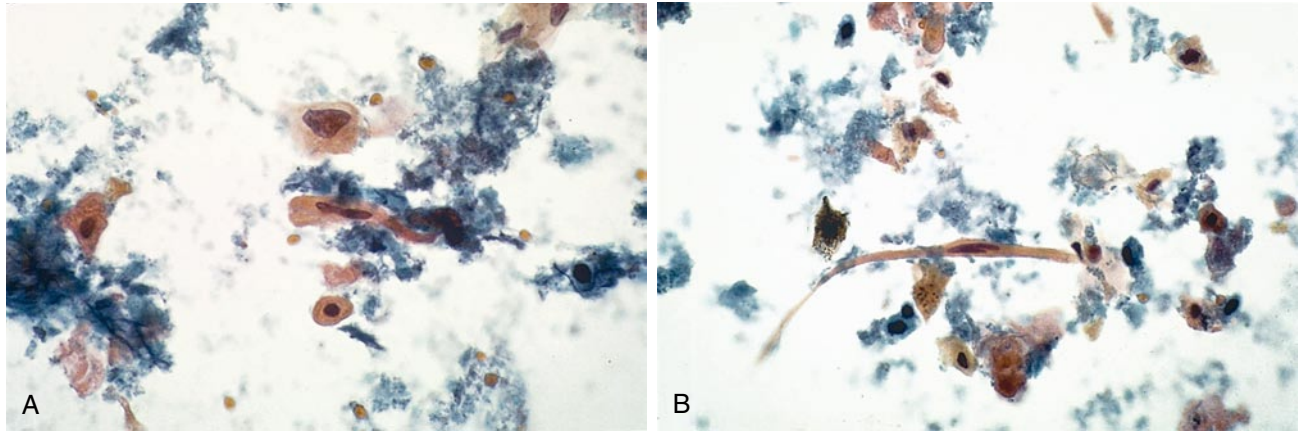


Figure 2.23 Squamous cell carcinoma ([SQC]; fine-needle aspiration [FNA]). A, Well-differentiated SQCs are composed of cells with dense, orangeophilic cytoplasm and hyperchromatic, often pyknotic nuclei, some with angular contours. B, Bizarre, elongated, spindle-shaped cells are common. Often there is abundant granular debris, seen here, and inflammation.

In moderately and poorly differentiated SQCs, keratinization is less apparent or absent altogether in the cytologic sample. The cytoplasm may retain the characteristic dense, smooth appearance of most SQCs, but in some poorly differentiated SQCs it is scant and less dense. The nuclei are larger and nucleoli are prominent. Chromatin, rather than smudgy, is coarsely textured and resembles the mottled and pitted surface of an Idaho potato (Fig. 2.24). The basaloid variant of SQC shows prominent palisading of nuclei around the perimeter of cell groups.

DIFFERENTIAL DIAGNOSIS OF SQUAMOUS CELL CARCINOMA:

- squamous metaplasia
- degenerative changes
- reactive squamous atypia (e.g., Aspergilloma)
- vegetable cells
- contamination from an upper airway cancer
- adenocarcinoma
- LCC
- small cell carcinoma
- metastatic SQC

Squamous metaplastic cells (from chronic irritation to the bronchial epithelium) are recognizably benign because of their small, round, normochromatic nuclei. In sputum samples, degenerative changes impart a pyknotic, condensed appearance to the nuclei of benign squamous cells that mimics the pyknotic, smudgy nuclei of SQC. Benign degenerated nuclei, however, are small and do not vary in size and shape as much as those of SQC. Reactive squamous cell atypias occur adjacent to cavitary fungal infections (Fig. 2.25) and stomas, and with almost any injury to the lung (e.g., infarction, radiation, chemotherapy, sepsis, and diffuse

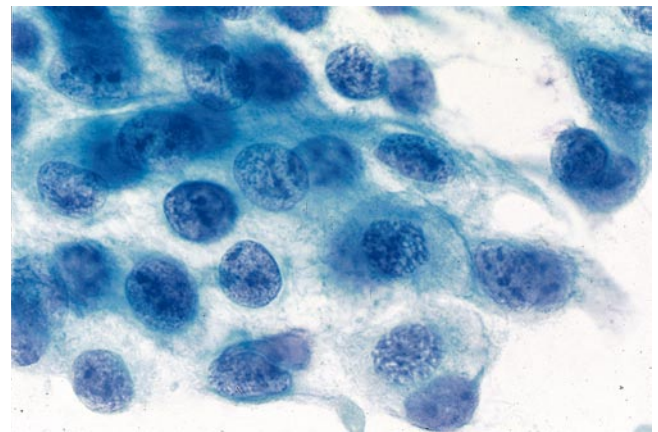


Figure 2.24 Squamous cell carcinoma ([SQC]; fine-needle aspiration [FNA]). Poorly differentiated squamous carcinoma cells are often arranged in thick groups of pulled out, spindled cells, rather than as dissociated cells. The nuclei are enlarged, and chromatin is coarsely granular, in contrast to the dense, frequently pyknotic nuclei of keratinizing SQCs. Often it is impossible to make a definitive diagnosis of SQC because of lack of differentiation or excessive group thickness. These difficult cases should be diagnosed as non-small cell carcinoma.

alveolar damage).^{33,34} To avoid a false-positive, caution is advised when the atypical squamous cells are few in number, poorly visualized, degenerated, or associated with a stoma, fungal infection, or any manner of severe lung injury. Vegetable food particles occasionally resemble keratinized squamous cells, but their cellulose wall and the regularity of their shape usually permit correct identification (see Fig. 2.12). Occasionally, malignant cells from an SQC of the upper airway may contaminate a specimen of the lower respiratory tract.⁶⁷

Most SQCs are easily distinguished from most adenocarcinomas of the lung based on the presence of obvious keratinization, mucin, or gland formation. When these tumors are poorly differentiated, however,

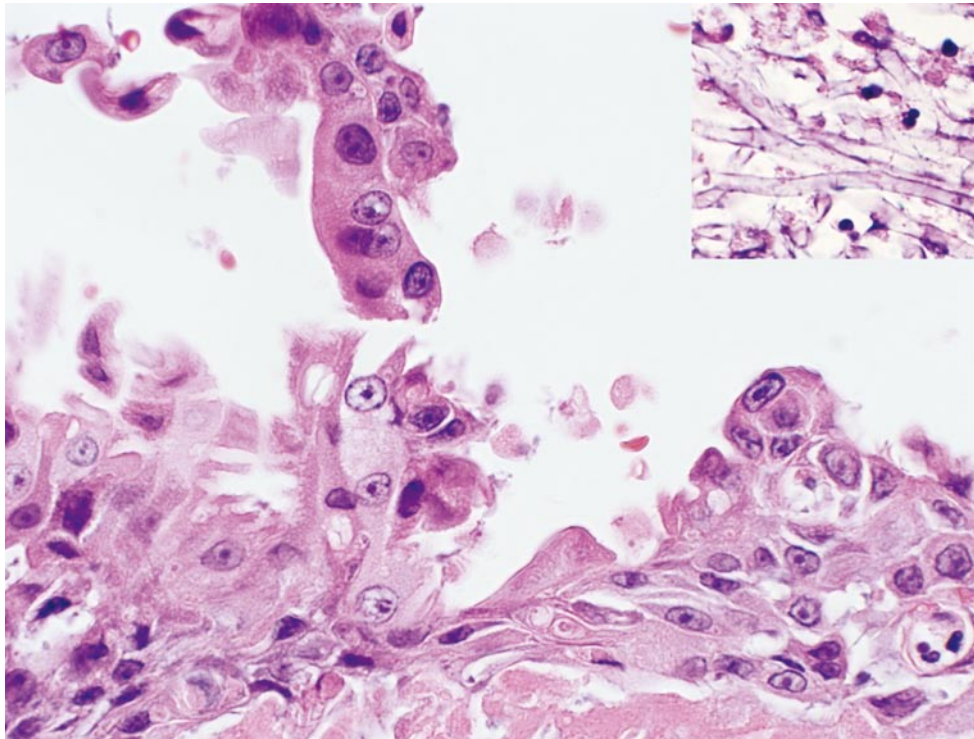


Figure 2.25 Atypical squamous metaplasia (fine-needle aspiration [FNA]). Cavitory fungal infections, such as this one by *Aspergillus* (inset), are among the causes of reactive squamous atypia, a mimic of squamous cell carcinoma (SQC).

the distinction becomes more difficult. In general, nuclear chromatin is more finely textured in adenocarcinomas and coarse in SQCs. The cytoplasm is thinner and more vacuolated in adenocarcinomas, and denser in SQCs. Histochemistry for mucin (mucicarmin and periodic acid-Schiff-D) and immunohistochemistry for p63 can be helpful. Although focal intracellular mucin can be seen in SQCs of the lung,¹²² more abundant mucin is diagnostic of adenocarcinoma, whereas immunoreactivity for p63 is typical of SQC. The possibility that both components are present (i.e., that the tumor might be an adenosquamous carcinoma) should be considered. If a definite distinction cannot be made, the interpretation “Non-small cell carcinoma” may be most appropriate. This applies equally well to those cases of poorly differentiated SQC that are difficult to distinguish from an LCC.

The small cell variant of SQC is comprised of smaller cells than the usual SQC. It is distinguished from small cell carcinoma because the cells of the small cell variant of SQC have coarse or pale chromatin, more prominent nucleoli, and distinct cell borders.

Metastatic SQCs are morphologically indistinguishable from primary SQCs of the lung. The distinction usually relies on clinical impression. In some cases, molecular studies can be helpful; testing for human papillomavirus can be helpful if there is a question of primary lung cancer versus metastatic SQC of the cervix.

Adenocarcinoma

Adenocarcinoma is the most common histologic subtype of lung cancer. The majority occur in the periphery of the lung and are often associated with a desmoplastic reaction and pleural puckering. Of all the histologic types, adenocarcinomas are most likely to be discovered incidentally in an asymptomatic individual. Some are detected based on clinically evident metastases. Adenocarcinomas rarely show cavitation.

The significant morphologic heterogeneity of pulmonary adenocarcinomas is reflected in the number of histologic variants recognized by the World Health Organization (WHO): acinar, papillary, bronchioloalveolar, solid with mucin production, fetal, mucinous (“colloid”), mucinous cystadenocarcinoma, and clear cell.¹²² The most common (80%) is the mixed subtype, which includes two or more of the histologic variants. The bronchioloalveolar subtype, commonly called bronchioloalveolar carcinoma (BAC), is defined by its growth along alveolar septa (lepidic growth) without destruction of the underlying alveolar architecture. There are two BAC subtypes: mucinous and nonmucinous. Metastatic cancers like pancreaticobiliary and colorectal adenocarcinomas can mimic a mucinous BAC.

The rare fetal adenocarcinoma resembles the epithelial component of the pulmonary blastoma.¹³⁸

Less than 10% of pulmonary adenocarcinomas harbor mutations of the EGFR, predominantly those in patients who are Asian and nonsmokers.¹³⁹⁻¹⁴¹ These mutations lie within the adenosine triphosphate (ATP)-binding pocket of this receptor tyrosine kinase (RTK) and result in its ligand-independent constitutive activation.^{142,143} Such mutations result in malignant transformation.¹⁴⁴ Because of the unique, extraordinary sensitivity of EGFR-mutated lung carcinomas to RTK small molecule inhibitors¹⁴⁵ like gefitinib or monoclonal antibodies directed against mutated EGFR, it is important to identify these tumors because the existence of the mutation entitles patients to this specialized and potentially less toxic treatment.^{146,147} Ultimately, resistance to RTK inhibitors by various mechanisms like point mutations¹⁴⁶ and Met oncogene amplification¹⁴⁸ is more the rule than the exception,¹⁴⁹ however, and more work is needed to develop lasting therapies. Cytologic preparations are suitable for EGFR mutation analysis,¹⁵⁰ and PCR methods like direct sequencing or the more sensitive Scorpions Amplified Refractory Mutation System (ARMS) have been shown to be effective (up to 91% sensitivity) in the identification of EGFR mutations in transbronchial FNA material.¹⁵¹ EGFR mutation-positive carcinomas are almost always adenocarcinomas,¹⁴⁰ with a tendency toward BAC morphology, but SQCs and other types have been described.¹⁵¹

CYTOMORPHOLOGY OF ADENOCARCINOMA:

- honeycomb-like sheets, three-dimensional clusters, acini, papillae
- eccentrically placed, round or irregular nuclei
- finely textured chromatin
- large nucleoli
- mucin vacuoles
- translucent, foamy cytoplasm

In cytologic preparations, adenocarcinoma cells can be arranged in a variety of architectural patterns: three-dimensional clusters, flat sheets, acini, and papillae (Figs. 2.26 and 2.27). The clustered cells tend to be loosely cohesive, such that isolated cells and small groups are also present. Cytoplasm is usually abundant, with well-defined borders. It can be thin and translucent or foamy. Some tumors have cells with a prominent large mucin vacuole. Nuclei are often eccentrically placed and usually have round, smooth contours, but in some tumors the nuclear membranes can be highly irregular. Chromatin is finely textured in most adenocarcinomas, but some poorly differentiated adenocarcinomas have coarsely textured chromatin. Nucleoli

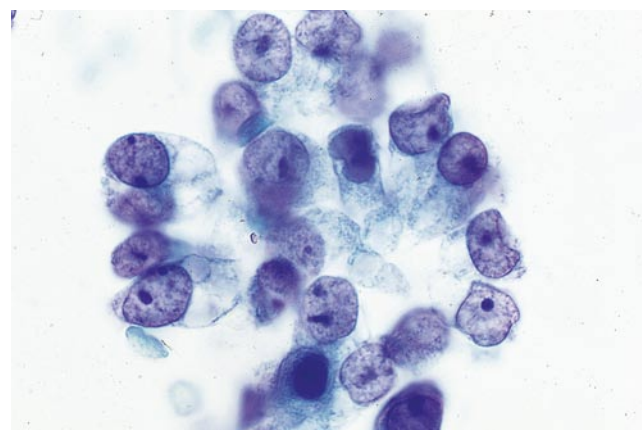


Figure 2.26 Adenocarcinoma (bronchial washing). The glandular differentiation can be easily appreciated. Cells are columnar, with polarized nuclei and single prominent nucleoli.

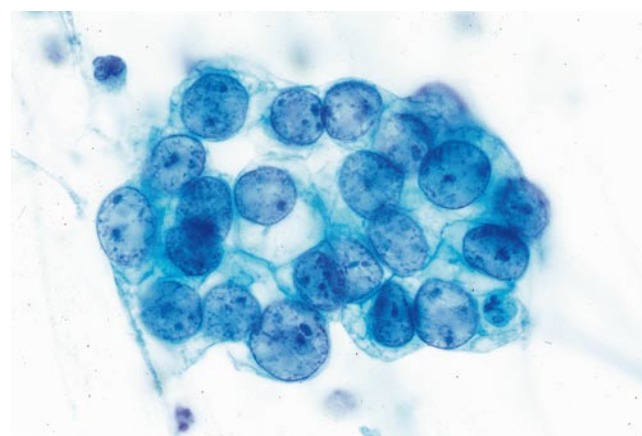


Figure 2.27 Adenocarcinoma (bronchial washing). These malignant cells have large, round nuclei with distinct nucleoli, open chromatin, and lacy, clear cytoplasm, and form a honeycomb-like arrangement.

are prominent in many adenocarcinomas. Psammoma bodies can be seen in the papillary and bronchioloalveolar variants.^{152,153}

Cytologic preparations from a mucinous BAC show uniform cells with pale, optically clear nuclei and inconspicuous nucleoli. Grooves and nuclear pseudoinclusions are often present (Fig. 2.28). In some cases, the cells of a mucinous BAC are dispersed and resemble macrophages. A nonmucinous BAC is indistinguishable from an ordinary adenocarcinoma on cytologic preparations (Fig. 2.29). Neither the mucinous nor the nonmucinous subtype of BAC can be diagnosed reliably on cytologic specimens, because the diagnosis is based on architecture: the absence of stromal, vascular, or pleural invasion.¹²² A mucinous BAC can be suspected, however, when characteristic cytology is correlated with imaging findings.^{122,154}

DIFFERENTIAL DIAGNOSIS OF ADENOCARCINOMA:

- reactive bronchial cells
- Creola bodies
- goblet cell hyperplasia
- reactive pneumocytes
- mesothelial cells (FNA specimens)
- vegetable cells
- hamartoma
- SQC
- large cell carcinoma
- small cell carcinoma
- epithelioid hemangioendothelioma and epithelioid angiosarcoma
- metastatic adenocarcinoma

Benign bronchial cell atypia and hyperplasia (anisonucleosis as a result of inflammation or injury, Creola bodies, goblet cell hyperplasia) can be recognized as a harmless mimic of adenocarcinoma if the atypical or hyperplastic cells have cilia (see [Figs. 2.4 and 2.5](#)) or demonstrate a spectrum of changes (from benign to markedly atypical). In a bronchial specimen, where malignant cells are often intermixed with benign bronchial cells, it is usually straightforward to identify two distinct cell populations, one malignant, the other benign.

The cells of a florid type II pneumocyte hyperplasia resemble those of adenocarcinoma (see [Fig. 2.8](#)). Attention to the clinical history (e.g., respiratory distress and diffuse infiltrates) can provide a clue that the cells are reactive. In a patient who is acutely ill with diffuse pulmonary infiltrates, markedly atypical cells should be interpreted with caution. Sequential respiratory specimens can help because hyperplastic pneumocytes are not present in BAL specimens more than a month after the onset of acute lung injury.³²

Mesothelial cells are common in percutaneous FNA specimens, and in some cases they can be numerous (see [Fig. 2.3](#)). They resemble the cells of a well-differentiated adenocarcinoma, particularly a mucinous BAC, but are recognized as benign mesothelial cells by their cohesion and the characteristic slitlike “windows” that separate them from each other. Some inadequate percutaneous FNAs contain only mesothelial cells. In such cases it is important to identify the sample as insufficient (nondiagnostic) rather than representative of any underlying lesion.

Vegetable cells and other contaminants can mimic the cells of an adenocarcinoma but are recognized as contaminants because of their prominent capsule.

Some hamartomas have a conspicuous glandular component that resembles that of a well or even moderately differentiated adenocarcinoma. Fragments of chondromyxoid matrix in the background are essential for establishing the diagnosis of a hamartoma and avoiding a false-positive interpretation.

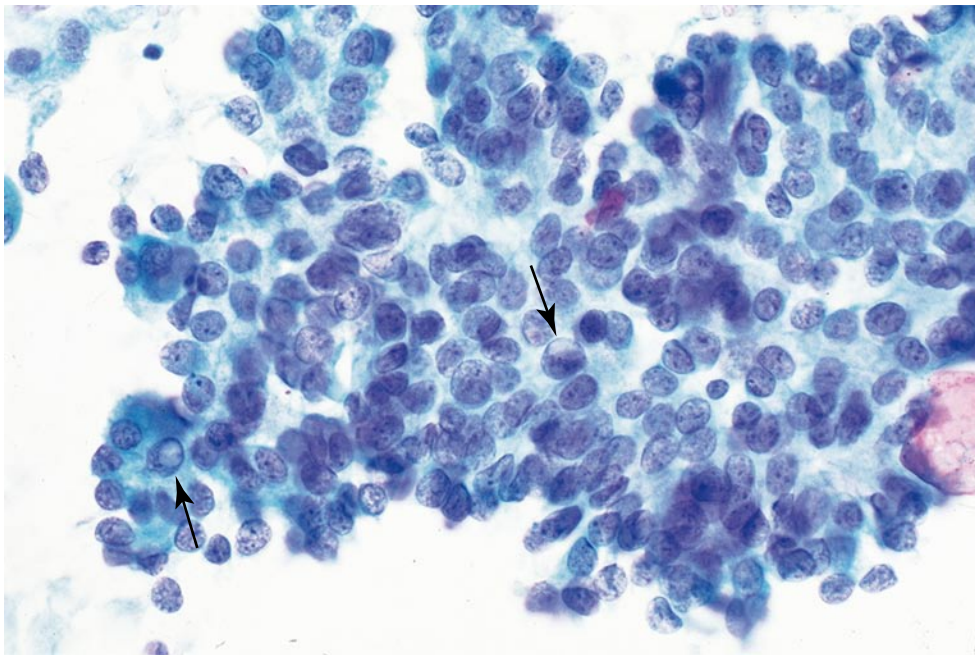


Figure 2.28 Bronchioloalveolar carcinoma, mucinous type (fine-needle aspiration [FNA]). The crowded sheets of these tumor cells, with their pale nuclei, small nucleoli, and occasional nuclear grooves, and intranuclear pseudoinclusions (arrows), are reminiscent of papillary carcinoma of the thyroid. Abundant extracellular mucin (not shown) is often present.

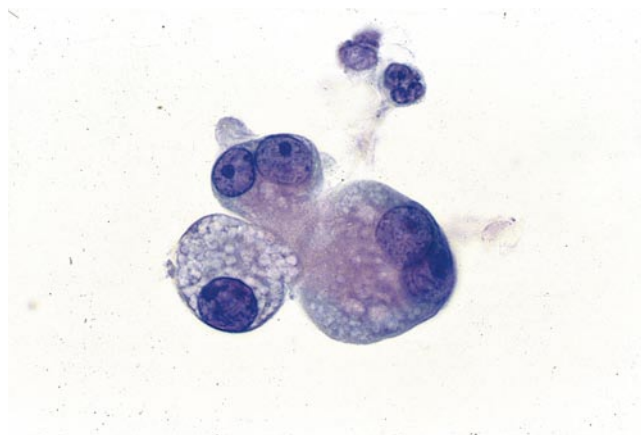


Figure 2.29 Bronchioloalveolar carcinoma, nonmucinous type (fine-needle aspiration [FNA]). These tumors are impossible to distinguish cytologically from a conventional adenocarcinoma.

Most adenocarcinomas are easily distinguished from SQCs because of obvious keratinization, mucin, or acinar formation. The cells of most adenocarcinomas are more cohesive than those of an SQC. When these tumors are poorly differentiated, however, the distinction becomes more difficult. In general, nuclear chromatin is more finely textured in adenocarcinomas and coarser in SQCs. The cytoplasm is thinner and more vacuolated in adenocarcinomas and denser in SQCs. A stain for mucin can be helpful: abundant mucin is diagnostic of an adenocarcinoma. The possibility that both components are present, (i.e., that the tumor might be an adenosquamous carcinoma) should be considered. If a definite distinction cannot be made, the interpretation “Non-small cell carcinoma” is appropriate. This applies equally well to those cases of poorly differentiated adenocarcinoma that are difficult to distinguish from an LCC.

The vascular tumors **epithelioid hemangioendothelioma (EHE)** and **epithelioid angiosarcoma (EAS)** occur as primary tumors in the lung and other sites. EHE is a low-to-intermediate grade tumor and EAS is high grade. They can present as a solitary mass or as multiple, bilateral pulmonary masses. Pleural involvement mimicking mesothelioma is not uncommon (see Fig. 4.8), and liver involvement is seen in 15% of cases. The cells of both tumors mimic carcinomas because they are epithelioid in shape. EHE is the more likely to be confused with adenocarcinoma because it is lower grade.¹⁵⁵ The common occurrence of intracytoplasmic vacuoles and intranuclear inclusions in EHE (and EAS) only adds to the confusion. The diagnosis of EHE and EAS is established by immunohistochemistry for vascular markers like CD31 and CD34. About 30% are reactive for keratins, however, and might be misinterpreted as carcinomas if a wider antibody panel is not used.

The differential diagnosis also includes metastatic adenocarcinoma. In a patient with a history of a previous neoplasm, it is helpful to compare the current specimen with the prior cytologic or histologic material to exclude a metastasis (see Fig. 2.39). Some cytologic features, such as the “dirty” necrosis and tall columnar cells of colorectal cancer, may suggest a specific primary site. Some metastatic tumors, however, are virtually indistinguishable from a primary lung adenocarcinoma. Metastatic papillary thyroid carcinoma mimics BAC to perfection: both tumors have intranuclear cytoplasmic pseudoinclusions, nuclear grooves, and optically clear nuclei. Immunohistochemistry can be essential. Adenocarcinomas of the lung are typically positive for CK7 and negative for CK20, and 75% express TTF-1. BAC is an exception, as it is often positive for CK20 and negative for TTF-1. Organ-specific antigens, such as thyroglobulin, prostate-specific antigen (PSA), Hepar1, and renal cell carcinoma antigen, are also helpful.

Large Cell Carcinoma

LCC is an undifferentiated non-small cell malignancy that accounts for about 9% of all lung cancers.¹²² It is a diagnosis of exclusion based on the absence of squamous, glandular, or small cell differentiation. Histologic variants include basaloid carcinoma, lymphoepithelioma-like carcinoma, clear cell carcinoma, LCC with rhabdoid phenotype, and large cell neuroendocrine carcinoma (LCNEC).¹²² Most tumors are located in the periphery of the lung, with the exception of the basaloid subtype. The neuroendocrine nature of the LCNEC is confirmed by immunohistochemical and ultrastructural studies.

CYTOMORPHOLOGY OF LARGE CELL CARCINOMA:

- syncytial clusters and dispersed cells
- irregular nuclei
- striking chromatin clearing
- prominent, often multiple nucleoli
- ill-defined, feathery cytoplasm

Cytologically, a diagnosis of malignancy is rarely difficult (Fig. 2.30). LCC cells are often arranged in large, syncytial-like sheets of crowded, overlapped cells. There is no apparent cytoplasmic differentiation. The nuclei are large and either round or markedly irregular, with irregularly distributed, coarse chromatin. Nucleoli are usually quite prominent. LCNEC shows nuclear palisading, nuclear molding, and rosettes^{156,157} (Fig. 2.31). Mitotic counts are high and there is usually necrosis. Confirmation of neu-

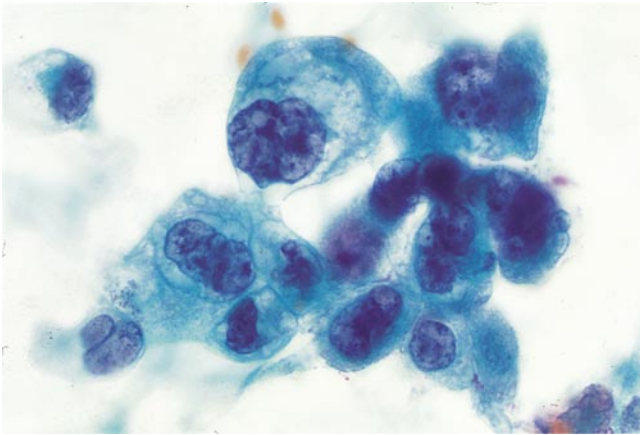


Figure 2.30 Large cell carcinoma (LCC; bronchial washing). The cells of this tumor are loosely arranged in aggregates. Nuclei are markedly enlarged with focal chromatin clearing and multiple irregular nucleoli.

roendocrine differentiation is required, with clear-cut reactivity for at least one of the neuroendocrine markers (synaptophysin, chromogranin, and CD56). Like LCNEC, **basaloid carcinoma** shows nuclear palisading around the margins of cell groups. The rare **lymphoepithelioma-like carcinoma** contains interspersed lymphoid cells. **Clear cell carcinoma** is comprised of large polygonal cells with abundant clear cytoplasm. The cells of **large cell carcinoma with rhabdoid features** have large cytoplasmic globules.

DIFFERENTIAL DIAGNOSIS OF LARGE CELL CARCINOMA:

- reactive changes (e.g., radiation reaction)
- adenocarcinoma
- SQC
- sarcomatoid carcinoma
- epithelioid angiosarcoma
- non-Hodgkin lymphoma
- metastatic carcinoma
- melanoma

Various types of lung injury (e.g., irradiation, infarction) can result in highly atypical bronchial cells or pneumocytes that mimic LCC and other tumors. The cells of a florid type II pneumocyte hyperplasia can be large and wildly pleomorphic, but most patients with this phenomenon are acutely ill with diffuse alveolar damage. In patients who are acutely ill or those with other injury to the lung, a conservative approach to diagnosis is recommended. When there is no question that the lesion is malignant, adenocarcinoma and SQC are unlikely if there is no keratinization or mucinous or glandular differentiation, but because the entire tumor has not been sampled, the possibility of an undersampled adenocarcinoma or SQC can never be entirely excluded. For this reason, cytologic specimens with features of LCC are often resulted as “Non-small cell carcinoma.” One of the

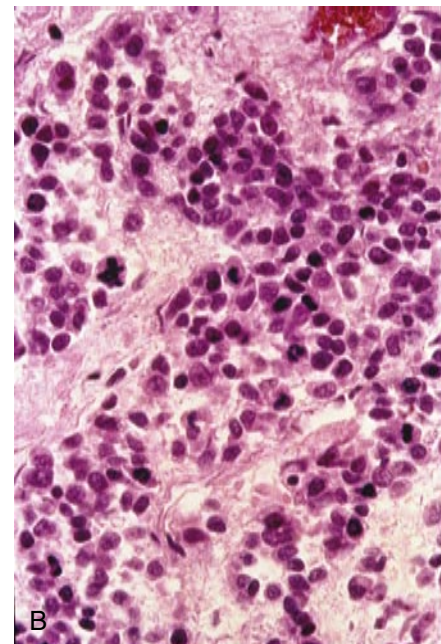
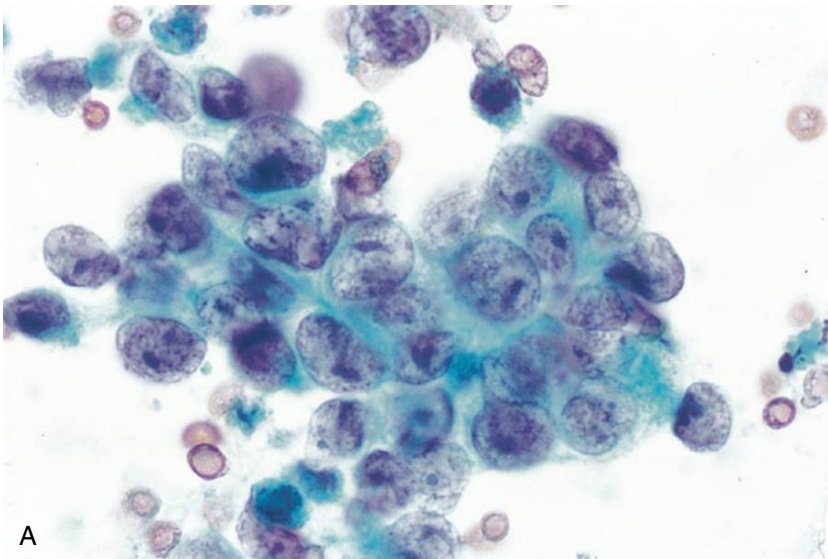


Figure 2.31 Large cell neuroendocrine carcinoma (LCNEC). A, Fine-needle aspiration (FNA). The key cytologic features include: rosettes, prominent nucleoli, and carcinoid tumor-like nuclei with extreme atypia and enlargement. Mitoses (*not seen*) are frequent. This constellation of findings is not always present, making distinction from non-small cell carcinoma sometimes impossible. B, Lung, autopsy specimen, same patient as A. Note the organoid growth pattern, rosettes, and brisk mitotic activity in the histologic material.

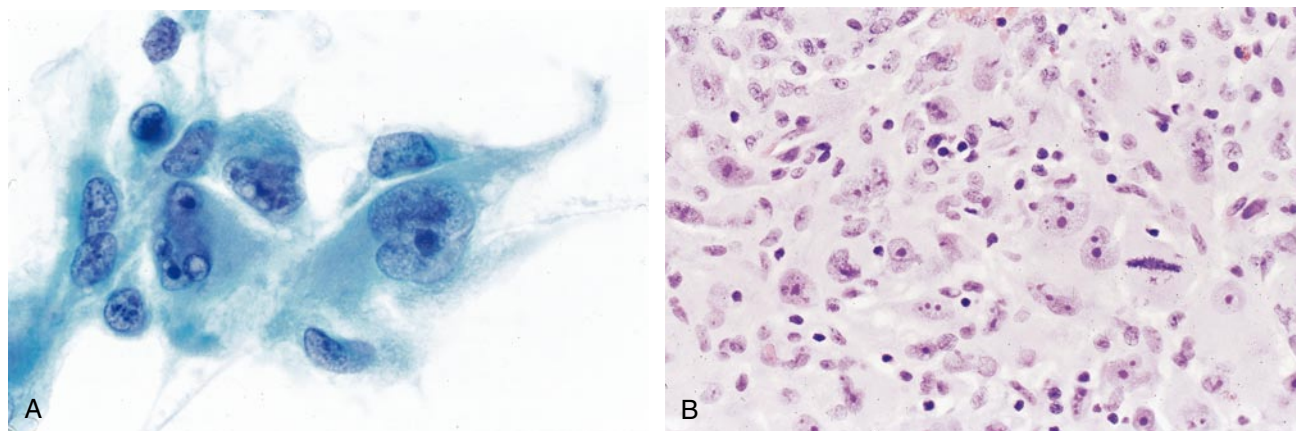


Figure 2.32 Epithelioid angiosarcoma (EAS) of the lung. A, The large polygonal tumor cells resemble those of a large cell carcinoma (LCC). B, Cell block sections show sheetlike growth.

sarcomatoid carcinomas should be considered if there is spindle or giant cell differentiation.

EAS occurs as a primary tumor in the lung and other sites. EAS is a particularly good mimic of LCC because it is a high-grade tumor, with large nuclei, prominent nucleoli, and brisk mitotic activity (Fig. 2.32). The diagnosis of EAS is established by demonstrating vascular differentiation with immunohistochemistry for CD31 or CD34. About 30% are reactive for keratins, however, and thus a wider antibody panel is essential.

Diffuse large B-cell lymphoma, anaplastic large cell lymphoma, metastatic carcinoma, and melanoma can usually be distinguished from LCC by an immunohistochemical panel that includes CD20, CD3, ALK1, S-100, HMB45, keratins CK7 and CK20, and carcinoma-specific markers selected based on clinical correlation.

Sarcomatoid Carcinoma

Sarcomatoid carcinoma is a family of poorly differentiated non-small cell carcinomas that show sarcomatoid or giant cell differentiation. The group includes pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma, and pulmonary blastoma. These tumors are important to recognize because they have a worse prognosis than the conventional non-small cell carcinomas.

Pleomorphic carcinoma is defined histologically as a poorly differentiated adenocarcinoma, SQC, or LCC, at least 10% of which is a spindle or giant cell malignancy. The spindle cell component shows no differentiated sarcomatous elements. Cytologic preparations show, in addition to adenocarcinoma, SQC, or LCC, a population of pleomorphic spindle or giant cells. Pleomorphic carcinoma is often a large peripheral tumor with a tendency to invade the chest wall.

Spindle cell carcinoma is a non-small cell carcinoma consisting only of spindle-shaped malignant cells with

no differentiated spindle cell elements. Cytologic preparations show large malignant spindle cells with hyperchromatic nuclei.

Giant cell carcinoma is composed of enormous, often multinucleated cells with no foci of adenocarcinoma, SQC, or LCC. Cytologic preparations show dispersed, isolated, strikingly large, often multinucleated cells with round nuclei and prominent nucleoli (Fig. 2.33). Neutrophils are often prominent. It is important to recognize giant cell carcinoma because it is associated with an aggressive clinical course.¹⁵⁸

The diagnosis of a pleomorphic, spindle cell, or giant cell carcinoma can be suspected on a cytologic specimen, but precise classification depends on extensive sampling and thus is best deferred to histologic examination.

The remaining two tumors are true biphasic neoplasms. **Pulmonary blastoma**, composed of primitive glandular and stromal elements, is a malignant neoplasm named for its resemblance to fetal lung. It is slightly more common in males, and the mean age is in the fourth decade.¹⁵⁹ Histologically there is a spindle cell component that can show myxoid, chondroid, osteoid, or rhabdomyoblastic differentiation and an epithelial component consisting of tubules of cuboidal to columnar cells with frequent mitoses and subnuclear and supranuclear vacuoles. The vacuoles contain glycogen and impart a “piano key”-like, endometrioid appearance to the epithelioid component. Solid nests of squamoid cells (squamoid morulas) are sometimes present. Tumors comprised of just the epithelial component of the pulmonary blastoma are called **fetal adenocarcinomas**.¹³⁸ Pulmonary blastoma most likely arises from a totipotent precursor because identical p53 mutations are present in both the epithelial and sarcomatous components within the same tumor.¹⁶⁰ Immunohistochemical demonstration of keratins in the epithelial component and muscle-specific actin in the spindle

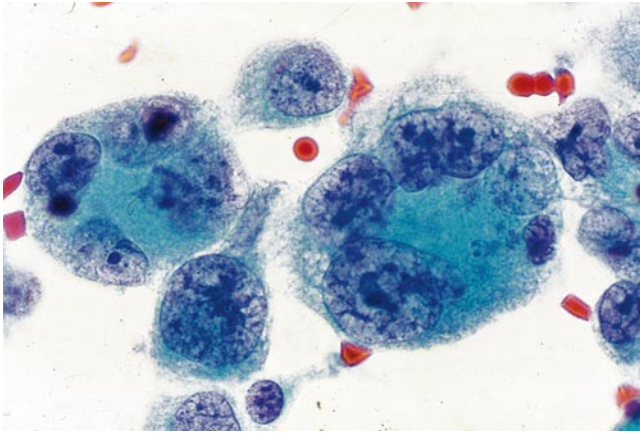


Figure 2.33 Giant cell carcinoma (fine-needle aspiration [FNA]). The huge multinucleated cells with striking nuclear atypia of this tumor are obviously malignant.

cells helps to confirm the diagnosis.¹⁶¹ Pitfalls in the cytologic diagnosis of blastoma occur when one of the components is poorly represented, as when the stromal component is misinterpreted as a small cell carcinoma.

Carcinosarcoma differs clinically from blastoma in the mean age of patients at presentation (65 rather than 40 years). Morphologically, the spindle cell component includes differentiated elements like malignant cartilage, bone, or skeletal muscle.

Pulmonary Neuroendocrine Neoplasms

This group of neoplasms encompasses a morphologic and biologic spectrum, divided by the World Health Organization into four distinct diagnostic categories: typical carcinoid, atypical carcinoid, LCNEC, and small cell carcinoma.¹²² By electron microscopy, all contain cytoplasmic dense-core, membrane-bound granules in variable quantities. Neuroendocrine tumors are immunoreactive for one or more of the neuroendocrine markers, chromogranin A (most specific), synaptophysin, and Leu-7 (CD57). Adrenocorticotrophic hormone (ACTH), insulin, calcitonin, gastrin, and vasoactive intestinal peptide are less consistently present.

The critical feature that separates typical and atypical carcinoids from LCNEC and small cell carcinoma is the mitotic rate (Table 2.3). Even if a tumor has morphologic features of small cell carcinoma, if it lacks frequent mitoses or necrosis, it should not be diagnosed as small cell carcinoma. LCNEC was previously discussed in greater detail.

Typical and atypical carcinoids are usually positive for keratins, but up to 20% can be negative.¹²² Conflicting results have been obtained with thyroid transcription factor 1 (TTF-1): carcinoids are either negative, or they can be positive in one third of cases.¹²²

Typical Carcinoid

At one end of the spectrum of neuroendocrine tumors is the typical carcinoid (TC), which accounts for 2% to 3% of all pulmonary tumors. Typical carcinoids are uniformly distributed throughout the lungs. Centrally located tumors are often submucosal, with a prominent endobronchial component. When submucosal, the typical carcinoid is usually covered by intact respiratory epithelium, and as a result sputum cytology is often negative. TCs have a low metastatic rate: 10% to 15% of patients have regional lymph node involvement,^{122,162} and 5% to 10% eventually metastasize to distant sites,¹²² but the prognosis is excellent with surgery, and 5-year survival is 90% to 98%.¹²²

Histologic examination reveals uniform polygonal cells with a variable growth pattern that may include nests, ribbons, papillae, and rosette-like arrangements. Occasionally the cells and nuclei are elongated (spindle cell carcinoid).¹⁶³

CYTOMORPHOLOGY OF TYPICAL CARCINOID:

- loosely cohesive groups and single cells
- rosette-like structures
- round, plasmacytoid, or elongated cells
- uniform nuclei with “salt and pepper” chromatin
- ample granular cytoplasm
- branching capillaries
- mitoses uncommon
- no necrosis

Cytologic preparations usually show a uniform population of isolated cells and loose clusters (Fig. 2.34). Large vascularized tissue fragments are sometimes present. TCs are vascular tumors, and sometimes a mesh of branching capillaries is encountered. In cell block sections, solid nests, trabeculae (ribbons), papillae, and rosettes can be appreciated. The tumor cells are round, oval (plasmacytoid), or elongated (spindle cell carcinoid) and have moderate or abundant granular cytoplasm. Nuclei are round or oval, with smooth contours, finely speckled (salt and pepper) chromatin, and inconspicuous nucleoli. In some cases there may be nuclear atypia and pleomorphism, but this has no clinical significance.

DIFFERENTIAL DIAGNOSIS OF TYPICAL CARCINOID:

- benign bronchial epithelial cells
- adenocarcinoma
- small cell carcinoma/atypical carcinoid
- lymphoma
- mesenchymal tumors

TABLE 2.3 CYTOLOGIC FEATURES OF PULMONARY NEUROENDOCRINE TUMORS*

	Typical Carcinoid	Atypical Carcinoid	Large Cell Neuroendocrine Carcinoma	Small Cell Carcinoma
Image				
Cell size	Small to medium	Medium	Large	Small to medium
Predominant pattern	Tight clusters/rosettes	Loose clusters/rosettes	Loose clusters/rosettes	Dispersed cells
Cytoplasm	Moderately abundant	Scant to moderate, lacy	Scant or moderate, lacy	Scant
Plexiform vascularity	Common	Common	Not known	Rare
Nuclear molding	Rare	Slight to moderate	Slight to moderate	Prominent
Chromatin	Coarsely granular	Coarsely granular	Coarsely granular	Finely granular
Nucleoli	Small	Occasionally prominent	Prominent	Inconspicuous
Mitoses (per 10 high power fields)	Rare (<2)	Uncommon (2–10)	Abundant (≥ 11 ; median 70)	Abundant (≥ 11 ; median 80)
Nuclear pleomorphism	Mild	Moderate	Marked	Moderate
Necrosis	Absent	Moderate	Marked	Marked
Nuclear crush	Absent	Mild	Moderate	Marked

*Cytologic features extrapolated from Travis WD, Linnoila RI, Tsokos MG, et al.: Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma: An ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. *Am J Surg Pathol* 1991;15:529–553, and authors' unpublished observations.

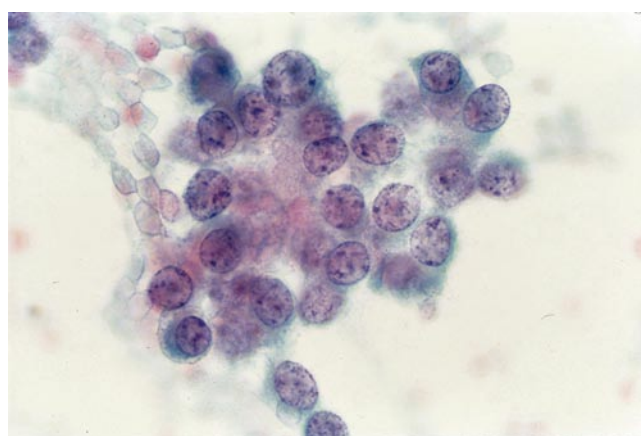


Figure 2.34 Typical carcinoid (fine-needle aspiration [FNA]). The tumor cells have a moderate amount of coarsely granular cytoplasm and a “salt-and-pepper” chromatin pattern. Rosettes are seen in some carcinoid tumors.

Because of their bland, monomorphous appearance, the cells of the typical carcinoid may be mistaken for benign bronchial cells. In contrast to bronchial cells, TC cells are less cohesive and lack cilia. Because rosettes are seen in some TCs, they are sometimes mistaken for an adenocarcinoma. In most adenocarcinomas, however, there is greater variation in cell size and more grouping of cells into spheres and sheets than with carcinoid tumors. Carcinoid tumors are distinguished from atypical carcinoids and small cell carcinomas by the lower mitotic rate and absence of necrosis (see Table 2.3). There may be a resemblance to lymphoid cells, but lymphoid cells have less cytoplasm and do not form clusters or rosettes.

Atypical Carcinoid

In the hazy area between the typical carcinoid and small cell carcinoma lies the atypical carcinoid (AT). It is more aggressive than the typical carcinoid: the 5-year survival rate is 61% to 73%.¹²²

CYTOMORPHOLOGY OF ATYPICAL CARCINOID:

- cells and architecture similar to typical carcinoid
- focal necrosis
- mitoses (moderate number)
- prominent nucleoli

The cytomorphologic features of the neuroendocrine tumors are listed in Table 2.3. ATs share many of the morphologic features of TCs (Fig. 2.35). They differ, however, in several slight ways: the architectural arrangements may be looser; there is greater (but not brisk) mitotic activity; there may be focal necrosis.¹⁶⁴ Although helpful, these features do not always permit precise classification in cytologic and bronchial biopsy samples. Precise classification depends on thorough histologic sampling of a resected specimen. In view of this, surgical excision should be considered for all localized neuroendocrine tumors.

Small Cell Carcinoma

Small cell carcinoma (SMC) accounts for 20% to 25% of all primary lung carcinomas, and 90% are centrally located. The majority of patients are male smokers

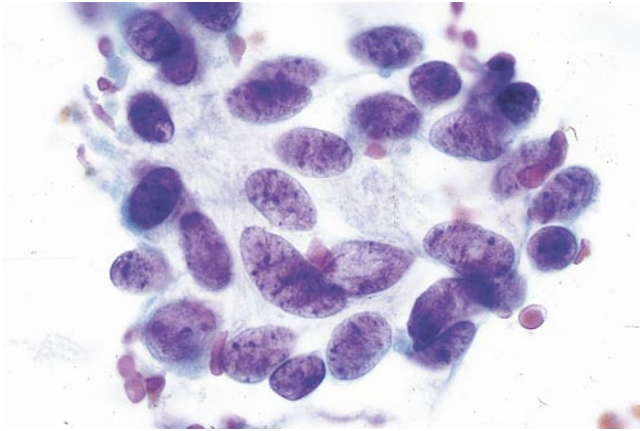


Figure 2.35 Atypical carcinoid (fine-needle aspiration [FNA]). All the features of typical carcinoid can be seen, but greater pleomorphism, slight nuclear enlargement, an increased number of mitoses, and focal necrosis are important distinguishing elements.

(80%), and their prognosis is poor (5-year survival <10%).¹⁶⁵ A morphologic variant is the “combined small cell carcinoma” (e.g., small cell/SQC, small cell/adenocarcinoma).

Histologically, SMC consists of small, round to fusiform cells with scant cytoplasm. The tumor often undermines the bronchial mucosa, with extensive invasion of lung parenchyma and lymphatics. Necrosis, a high mitotic rate, and nuclear encrustation of vascular walls are characteristic features. In some cases the cells are larger, with more cytoplasm and vesicular nuclei.

CYTOMORPHOLOGY OF SMALL CELL CARCINOMA:

- small cells (twice the size of lymphocytes), occasionally larger
- carrot-shaped nuclei
- evenly dispersed, powdery chromatin
- nuclear molding
- small to indistinct nucleoli
- paranuclear blue bodies
- mitoses
- scant cytoplasm
- background of nuclear debris and crush artifact

Cytologically, the cells are loosely aggregated and dispersed as isolated cells. The cells of SMC are fragile. Bare nuclei stripped of cytoplasm and crush artifact with smearing of nuclear DNA (chromatin streaks) are common. Crush artifact can be so marked that mitoses, which are abundant but delicate, may all be smeared and not identifiable. In such cases, apoptotic cells and single cell necrosis are considered evidence of rapid cell turnover and abundant mitotic activity. Depending on the tumor, the cells may be small or

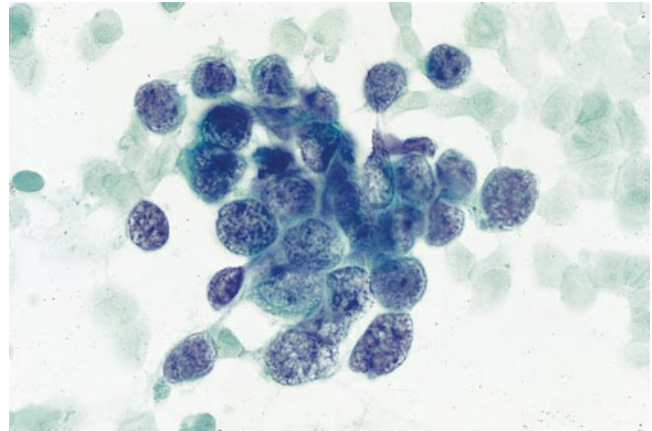


Figure 2.36 Small cell carcinoma (SMC; bronchial brushing). The cells of SMC are tightly packed and show nuclear molding. They are fragile and occasionally degenerated. Well-preserved cells have a powdery chromatin texture.

intermediate in size. Cytoplasm is scant and inconspicuous, and adjacent nuclei show frequent molding (Fig. 2.36). The cytoplasm can contain occasional *paranuclear blue bodies*, solitary round, light to dark blue, homogeneous spheres that indent the nucleus.¹⁶⁶ Nuclei are round, oval, or stretched out (carrot-shaped). Chromatin is finely textured, and nucleoli are absent or uncommon. The cytologic appearance varies somewhat with the sampling method. In sputum, streams of small hyperchromatic cells form loose aggregates. In bronchial brushings and FNA specimens, the cells are better preserved.

DIFFERENTIAL DIAGNOSIS OF SMALL CELL CARCINOMA:

- reserve cell hyperplasia
- lymphocytes
- typical carcinoid
- atypical carcinoid
- non-small cell carcinomas
- Ewing sarcoma
- neuroblastoma
- Wilms tumor
- rhabdomyosarcoma
- pulmonary blastoma

The cells of reserve cell hyperplasia can show molding, but they are smaller and more cohesive than those of SMC; there is no necrosis, mitoses are absent, and nuclei have smudged, featureless chromatin (see Fig. 2.7). Lymphoid cells, whether from an inflammatory process, an intrapulmonary lymph node, or lymphoma, can be mistaken for SMC. Lymphoid cells generally do not form cell clusters, except artifactually on cytospin or thinlayer preparations; they tend to be evenly spaced

rather than molded together; and they are one-half the size of SMC cells.

The distinction between an SMC and a TC is usually straightforward, given that TC lack nuclear molding, necrosis, and mitotic activity. In addition, the chromatin texture of TC is more coarsely granular than that of SMC. Some TC, like the spindle cell variant, however, bear a superficial resemblance to SMC. Therefore, care must be taken not to make a diagnosis of SMC without abundant mitotic activity and necrosis. The distinction between TC and AC (see Fig. 2.35), and between AC and SMC, is more difficult; distinguishing features are summarized in Table 2.3. In general, ACs have more numerous mitoses than TCs, and SMCs have significantly more mitoses than either TCs or ACs, but a final determination may not be possible without thorough histologic sampling.

SMC is sometimes difficult to distinguish from the non-small cell carcinomas, particularly the small cell variant of SQC and those adenocarcinomas composed of relatively small cells. The most helpful features in making this distinction are the powdery chromatin texture, inconspicuous nucleoli, prominent nuclear molding, and scant cytoplasm of SMC. Care should be taken not to misinterpret the solid paranuclear blue bodies as mucin vacuoles (which are clear), otherwise an SMC might be misidentified as an adenocarcinoma.¹⁶⁶ Immunohistochemistry for p63 can be quite helpful in distinguishing SQC, which is immunoreactive, from SMC, which is negative.^{167,168} Even after careful examination and immunohistochemical study, not all tumors are easily classified. In such cases, the possibility of a mixed tumor, a common occurrence in the lungs, should be considered.

Clinical history, particularly patient age, provides clues to the diagnosis of the other small round cell tumors. The sarcomatous component of pulmonary blastomas resembles a SMC; identifying the epithelial areas is crucial in making this distinction.

UNCOMMON PULMONARY TUMORS

Adenoid Cystic Carcinoma and Other Bronchial Gland Tumors

The submucosal glands of the trachea and bronchi give rise to neoplasms that resemble those occurring in the salivary glands. The most common of these is the **adenoid cystic carcinoma**. This malignant tumor accounts for 20% to 35% of all cancers in the trachea but also occurs in the major bronchi. The tumors grow as polypoid masses, undermining intact overlying respiratory epithelium. They often infiltrate the cartilage and soft tissues of the major airways. Symptoms are related to obstruction, and

include cough, dyspnea, and hemoptysis. Treatment is surgical, but the long-term prognosis is poor, with 80% of patients dead of disease within 20 years.¹⁶⁹

These tumors are most often diagnosed by means of bronchial brushings or transbronchial needle aspiration. Cytologic preparations show cylinders or spheres of small, deceptively innocuous-appearing epithelial cells that surround basal lamina material (see Figs 10.20 to 22).

Mucoepidermoid carcinomas of the trachea and bronchi are uncommon, representing only 0.2% of lung tumors. They are defined by the presence of squamous, glandular, and intermediate cells, and by their origin within cartilage-containing airways. The diagnosis “adenosquamous carcinoma” should be restricted to tumors arising at the lung periphery beyond a recognizable bronchus. Mucoepidermoid carcinomas can develop in persons of all ages, including children. They are endobronchial tumors like those of adenoid cystic carcinoma and other salivary gland tumors, and the presenting symptoms are similar. Cytologically, the tumors are diagnosed by finding squamous cells, intermediate cells, and mucinous cells (see Figs 10.17, 10.18). High-grade tumors show marked nuclear atypia. Mucoepidermoid carcinoma is another one of a growing number of neoplasms for which a recurrent and tumor-specific genetic aberration is present: the t(11;19), forming the MECT1-MAML2 fusion transcript.¹⁷⁰

Other bronchial gland tumors include pleomorphic adenoma, acinic cell carcinoma, and oncocytoma.

Clear Cell Tumor (“Sugar Tumor”)

The clear cell tumor is extremely rare and can occur in persons of all ages, most of whom are asymptomatic. It is usually a peripheral mass and ranges from 1 to 7 cm in greatest diameter. Clear cell tumors most likely arise from perivascular epithelioid cells.^{122,171} Histologically and cytologically, the tumor consists of bland polygonal and spindle-shaped cells with a central oval or elongated nucleus and clear, glycogen-rich cytoplasm.¹⁷² The differential diagnosis includes the clear cell variant of adenocarcinoma or SQC of the lung, granular cell tumor, and metastatic tumors with clear-cell features (renal cell carcinoma, adrenal cortical carcinoma, some melanomas, and others).^{172,173} Immunohistochemistry is helpful for most of these distinctions except with melanoma: most clear cell tumors are positive for HMB-45 and Melan-A and negative for cytokeratins and CEA.^{171,174}

Sarcomas

Primary malignant mesenchymal tumors of the lung are rare, occurring in adults of either sex. They can be large masses or small endobronchial lesions. One of the

most common primary sarcomas is leiomyosarcoma.^{175,176} Others include fibrosarcoma, solitary fibrous tumor, chondrosarcoma,¹⁷⁷ Kaposi sarcoma, angiosarcoma,¹⁷⁸ epithelioid hemangioendothelioma,¹⁵⁵ rhabdomyosarcoma, malignant peripheral nerve sheath tumor, and synovial sarcoma. Care must be taken not to mistake stromal and smooth muscle cells in ulcerative processes for sarcoma cells. Most sarcomas, both primary and metastatic, can confidently be distinguished from carcinomas on FNA smears.^{179,180}

CYTOMORPHOLOGY OF SARCOMAS:

- sheetlike, cellular aggregates
- single, highly atypical spindle cells

DIFFERENTIAL DIAGNOSIS OF SARCOMAS:

- spindle cell carcinoma
- spindle cell carcinoid
- spindle cell thymoma
- sarcomatoid mesothelioma
- melanoma

Whether the sarcoma is primary or metastatic is a distinction made mainly on clinical grounds. Immunohistochemistry for keratins, CEA, desmin, smooth muscle actin, CD31, CD34, calretinin, WT-1, S-100, and HMB45 can be quite useful in the differential diagnosis.

Lymphomas and Leukemias

Hodgkin and non-Hodgkin lymphomas occur as primary tumors of the lung, but spread from an extrapulmonary primary is more common. Primary pulmonary non-Hodgkin lymphoma represents fewer than 10% of all extranodal lymphomas. By contrast, secondary lung involvement by non-Hodgkin lymphomas is noted in 20% to 50% of patients at some point in their clinical course.¹⁸¹ The most common primary non-Hodgkin lymphoma of the lung (70% to 90%) is *extranodal marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma)*, followed by *diffuse large B-cell lymphoma*. Much less common primary lymphoproliferative disorders include *lymphomatoid granulomatosis* and *peripheral T cell lymphoma*.¹⁸² MALT lymphomas are typically incidental solitary pulmonary masses and have an indolent clinical course: the 5-year survival rate is around 90%.^{122,182} By contrast, lymphomatoid granulomatosis, an Epstein-Barr virus (EBV)-associated, T-cell-rich B-cell lymphoproliferative disorder, is often associated with chest pain, cough, dyspnea, and neurologic symptoms,^{183,184} and the median survival is 2 years.

DIFFERENTIAL DIAGNOSIS OF PULMONARY LYMPHOMA AND LEUKEMIA:

- inflammatory lesions
- small cell carcinoma
- LCC
- melanoma

MALT lymphoma can be particularly troublesome to diagnose by cytology alone because it is indistinguishable from a reactive condition (Fig. 2.37). Identifying a diffuse large B-cell lymphoma is much more reliable because of the striking atypia of the large cells. Surface marker analysis to demonstrate monoclonality, best determined by flow cytometric analysis¹⁸⁵ is often essential to confirm the diagnosis of a MALT lymphoma. A diffuse large B-cell lymphoma, which is obviously cytologically malignant, need only stain for lymphoid markers (e.g., CD20 or leukocyte common antigen)¹⁸⁶ for diagnostic confirmation.

Primary pulmonary Hodgkin lymphoma is exceptionally rare, though secondary involvement occurs in 40% to 50% of patients.^{181,187} Primary pulmonary involvement is two-fold more common in women, who present with cough and B-type symptoms. Radiologic findings are equally divided among solitary lesions, multiple masses, and a pneumonia-like infiltrate. Patients who present with pulmonary involvement have a poorer prognosis than those with nodal disease only. The cytologic diagnosis of Hodgkin lymphoma rests on identifying Reed-Sternberg cells.¹⁸⁷ Mononuclear variants and a mixed background of lymphocytes, plasma cells, eosinophils, and histiocytes are typically present.

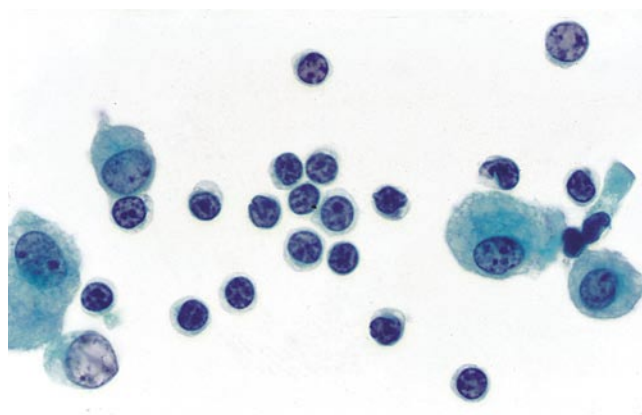


Figure 2.37 Lymphocytic interstitial pneumonia (bronchoalveolar lavage [BAL]). This demonstrates a polymorphous population comprised of small- and medium-sized lymphocytes. Although this cytologic pattern suggests a reactive process, a mucosa-associated lymphoid tissue (MALT) lymphoma cannot be excluded. Immunophenotyping is essential to confirm the reactive or neoplastic nature of the lesion.

Granulomatous inflammatory processes need to be distinguished from Hodgkin lymphoma. Histiocytes can mimic Reed-Sternberg cells, and granulomas can occur in Hodgkin lymphoma. Reed-Sternberg cells are distinguished from histiocytes because RS cells have large, inclusion-like nucleoli and highly irregular nuclei. Melanoma and LCC can be excluded with immunohistochemical studies for keratins, S-100, and HMB45.

About 10% of diffuse pulmonary infiltrates in patients with leukemia result from leukemic involvement of the lung. At autopsy, infiltrates are seen in 25% to 65% of cases, most commonly acute leukemia. Leukemic involvement of the lung may also present as a mass lesion. The cells are dispersed as isolated cells, as with lymphomas, and cellular monomorphism is characteristic. BAL is useful for demonstrating leukemic involvement, especially when biopsy is contraindicated because of coagulopathy.¹⁸⁸ The diagnosis can be confirmed using immunohistochemistry on cell block, cytospin preparations, or by flow cytometry (i.e., blasts are commonly CD34+).

Other hematopoietic malignancies that can involve the lung include myeloma¹⁸⁹ and mycosis fungoides.¹⁹⁰

METASTATIC CANCERS TO THE LUNG

The lungs receive the entire cardiac output, so it is not surprising that they are a common site for metastasis; 30% to 50% of extrapulmonary cancers involve the lungs at autopsy.¹⁹¹

Metastases are diagnosed in sputum in up to 70% of cases.¹⁹² About 40% of metastatic tumors in exfoliative specimens are squamous carcinomas from an adjacent site in the aerodigestive tract. The remainder of the cases includes adenocarcinomas (34%)—most commonly of the breast, kidney, and colon—and hematopoietic malignancies (8%).¹⁹³

Because confirming metastatic disease is a major indication for transthoracic FNA, metastatic tumors account for between 14% and 26% of transthoracic FNA specimens.¹⁹⁴ In a study of more than 1000 cases, malignant melanoma accounted for 27%, urinary and male genital tract neoplasms for 17%, breast carcinoma for 15%, female genital tract neoplasms for 13%, and gastrointestinal tract neoplasms for 10%.¹⁹³ Another study reported that the most common metastases (in decreasing frequency) are those from breast cancer, colon cancer, renal cell carcinoma, bladder cancer, and melanoma.¹⁹⁵

Primary sites for some metastatic carcinomas can be inferred based on their characteristic cytologic features. The cells of the usual type of colon carcinoma have a distinctive tall, columnar “picket fence” appearance, with hyperchromatic nuclei and “dirty” necrosis. Malignant melanoma often demonstrates a single-cell arrangement, with cytoplasmic pigment, intranuclear cytoplasmic invaginations, and classic “mirror-image” binucleation. Breast carcinoma often shows a single-file arrangement of cells, some with mucin-containing intracellular lumens (Fig. 2.38); renal cell carcinoma cells are large cells with abundant clear cytoplasm and poorly defined cell membranes; and metastatic papillary thyroid carcinomas have

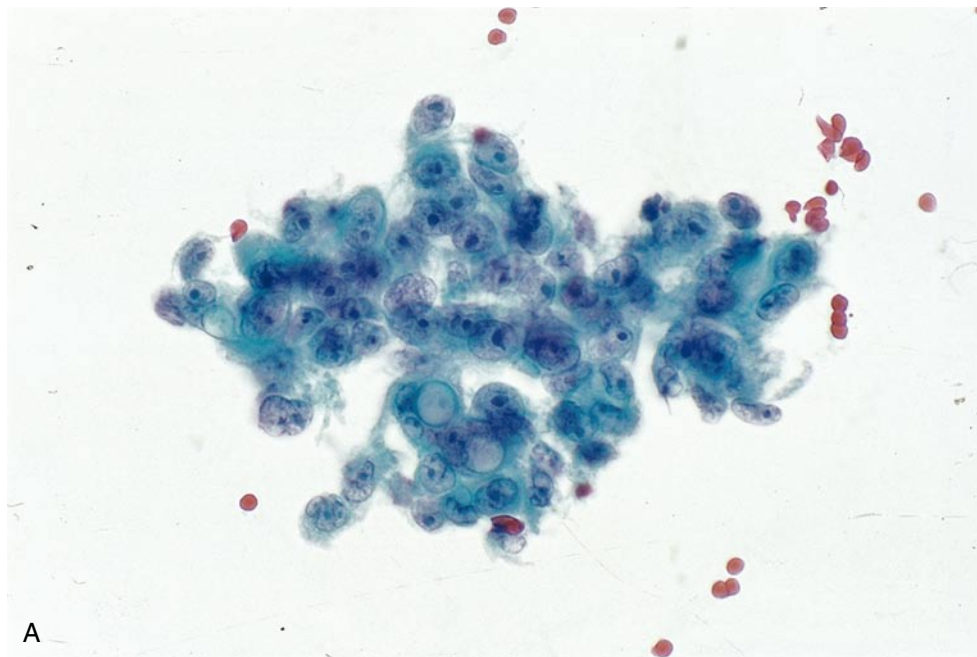


Figure 2.38 Metastatic ductal carcinoma of the breast. This patient had a previous history of ductal breast carcinoma. A, Fine-needle aspiration (FNA) of the lung. The smear shows clusters of tumor cells with prominent nucleoli and mucin-filled intracellular lumens.

Illustration continued on following page

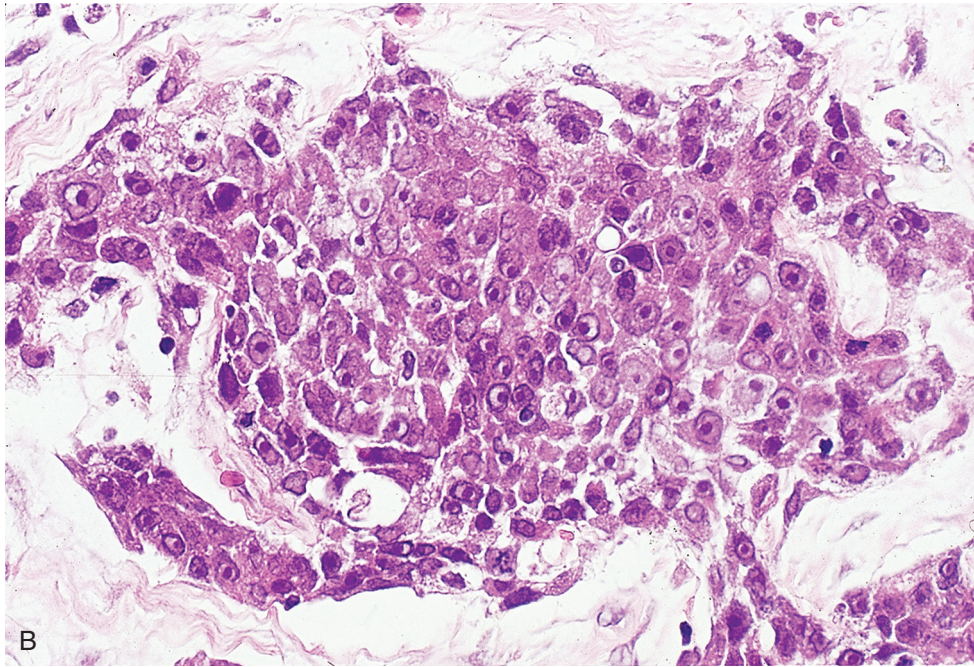


Figure 2.38 (Continued) **Metastatic ductal carcinoma of the breast.** B, Breast lumpectomy. Comparison with the previously resected tumor shows close similarity, supporting the diagnosis of metastasis to the lung from the breast primary.

nuclear grooves, nuclear pallor, and intranuclear pseudoinclusions, a morphologic appearance mimicked by the mucinous type of bronchioloalveolar carcinoma.

The distinction of primary lung carcinoma from a metastasis is often facilitated by immunohistochemistry. Most lung carcinomas are reactive for CK7 and nonreactive for CK20 (as contrasted with metastatic colon cancer, for example, which is CK7- and CK20+). Additionally, lung carcinomas can be distinguished from non-lung carcinomas, except those of thyroid origin, with antibodies to thyroid transcription factor 1.¹⁹⁶ Interestingly, whereas thyroid transcription factor 1 was once thought to be simply a marker of lung origin, there is now evidence that it is involved in the pathogenesis of lung cancer: 12% of lung carcinomas have amplification of thyroid transcription factor 1.¹⁹⁷

Determining the primary site of the metastasis (see Fig. 2.38) is greatly aided by comparing the patient's cytologic specimen with any previous pathologic material. It is particularly important to obtain sufficient cytologic material for cell block preparations so that tissue architecture and immunohistochemical markers can be assessed.

REFERENCES

1. Johnston WW, Frable WJ: Diagnostic Respiratory Cytopathology. New York, Masson Publishing, 1979.
2. Linder J: Lung cancer cytology. Something old, something new. *Am J Clin Pathol* 2000;114:169-171.
3. Dahlgren S, Nordenstrom B: *Transthoracic Needle Biopsy*. Chicago: Year Book Medical Publishers, 1966.
4. Johnston WW: Ten years of respiratory cytopathology at Duke University Medical Center. III. The significance of inconclusive cytopathologic diagnoses during the years 1970 to 1974. *Acta Cytol* 1982;26:759-766.
5. Wax TD, Coogan AC, Johnston WW: Clinical significance of an inconclusive cytopathologic diagnosis: A five-year experience at Duke University Medical Center. II. Analysis and follow-up of specimens from the respiratory tract. *Acta Cytol* 1997;41(4):1053-1057.
6. Guidelines of the Papanicolaou Society of Cytopathology for the examination of cytologic specimens obtained from the respiratory tract. The Papanicolaou Society of Cytopathology Task Force on Standards of Practice. *Mod Pathol* 1999;12(4):427-436.
7. Khajotia RR, Mohn A, Pokieser L, et al.: Induced sputum and cytological diagnosis of lung cancer. *Lancet* 1991;338:976-977.
8. Saccomanno G, Saunders RP, Ellis H, et al.: Concentration of carcinoma or atypical cells in sputum. *Acta Cytol* 1963;7:305-310.
9. Tang CS, Tang CM, Lau YY, Kung IT: Sensitivity of sputum cytology after homogenization with dithiothreitol in lung cancer detection. Two years of experience. *Acta Cytol* 1995;39(6):1137-1140.
10. Böcking A, Biesterfeld S, Chatelain R, et al: Diagnosis of bronchial carcinoma on sections of paraffin-embedded sputum: Sensitivity and specificity of an alternative to routine cytology. *Acta Cytol* 1992;36:37-47.
11. Erozan YS, Frost JK: Cytopathologic diagnosis of cancer in pulmonary material: A critical histopathologic correlation. *Acta Cytol* 1970;14:560-565.
12. Fraire AE, Underwood RD, McLarty JW, Greenberg SD: Conventional respiratory cytology versus fine needle aspiration cytology in the diagnosis of lung cancer. *Acta Cytol* 1991;35(4):385-388.
13. Rosenthal DL, (ed): *Cytopathology of Pulmonary Disease*. Basel, S. Karger, 1988.
14. Sing A, Freudenberg N, Kortsik C, et al.: Comparison of the sensitivity of sputum and brush cytology in the diagnosis of lung carcinomas. *Acta Cytol* 1997;41(2):399-408.

15. Suprun H, Pedio G, Ruttner JR: The diagnostic reliability of cytologic typing in primary lung cancer with a review of the literature. *Acta Cytol* 1980;24:494-500.
16. Liang XM: Accuracy of cytologic diagnosis and cytotyping of sputum in primary lung cancer: Analysis of 161 cases. *J Surg Oncol* 1989;40:107-111.
17. Pue CA, Pacht ER: Complications of fiberoptic bronchoscopy at a university hospital. *Chest* 1995;107:430-432.
18. Popp W, Rauscher H, Ritschka L, et al.: Diagnostic sensitivity of different techniques in the diagnosis of lung tumors with the flexible fiberoptic bronchoscope: Comparison of brush biopsy, imprint cytology of forceps biopsy, and histology of forceps biopsy. *Cancer* 1991;67:72-75.
19. Kawan E, Ulrich W, Redtenbacher S, et al.: Kawan bronchial brush/cellblock technique. Facilitation of the routine diagnosis of bronchial neoplasms. *Acta Cytol* 1998;42:1409-1413.
20. Raab SS, Oweity T, Hughes JH, et al.: Effect of clinical history on diagnostic accuracy in the cytologic interpretation of bronchial brush specimens. *Am J Clin Pathol* 2000;114(1):78-83.
21. Johnston WW: Ten years of respiratory cytopathology at Duke University Medical Center. I: The cytopathologic diagnosis of lung cancer during the years 1970 to 1974, noting the significance of specimen number and type. *Acta Cytol* 1981;25:103-107.
22. Mak VH, Johnston ID, Hetzel MR, Grubb C: Value of washings and brushings at fiberoptic bronchoscopy in the diagnosis of lung cancer. *Thorax* 1990;45:373-376.
23. Kahn FW, Jones JM: Diagnosing bacterial respiratory infection by bronchoalveolar lavage. *J Infect Dis* 1987;155(5):862-869.
24. Pisani RJ, Wright AJ: Clinical utility of bronchoalveolar lavage in immunocompromised hosts. *Mayo Clin Proc* 1992;67:221-227.
25. Broaddus C, Dake MD, Stulbarg MS, et al.: Bronchoalveolar lavage and transbronchial biopsy for the diagnosis of pulmonary infections in the acquired immunodeficiency syndrome. *Ann Intern Med* 1985;102:747-752.
26. Baughman RP, Dohn MN, Frame PT: The continuing utility of bronchoalveolar lavage to diagnose opportunistic infection in AIDS patients. *Am J Med* 1994;97(6):515-522.
27. Atzori C, Angeli E, Mainini A, et al.: In vitro activity of human immunodeficiency virus protease inhibitors against *Pneumocystis carinii*. *J Infect Dis* 2000;181(5):1629-1634.
28. Piller CF, Clark DP: Pathologic outcome in HIV-seropositive individuals with nonspecific bronchoalveolar lavage cytology. *Acta Cytol* 1998;42:913-917.
29. Pirozynski M: Bronchoalveolar lavage in the diagnosis of peripheral, primary lung cancer. *Chest* 1992;102(2):372-374.
30. Rennard SI: Bronchoalveolar lavage in the diagnosis of cancer. *Lung* 1990;168(Suppl):1035-1040.
31. Semenzato G, Poletti V: Bronchoalveolar lavage in lung cancer. *Respiration* 1992;9(Suppl 1):44-46.
32. Stanley MW, Henry-Stanley MJ, Gajl-Peczalska KJ, Bitterman PB: Hyperplasia of type II pneumocytes in acute lung injury: Cytologic findings of sequential bronchoalveolar lavage. *Am J Clin Pathol* 1992;97:669-677.
33. Gulbahce HE, Baker KS, Kumar P, et al.: Atypical cells in bronchoalveolar lavage specimens from bone marrow transplant recipients. A potential pitfall. *Am J Clin Pathol* 2003;120(1):101-106.
34. Policarpio-Nicolas ML, Wick MR: False-positive interpretations in respiratory cytopathology: Exemplary cases and literature review. *Diagn Cytopathol* 2008;36(1):13-19.
35. Herth FJ, Becker HD, Ernst A: Ultrasound-guided transbronchial needle aspiration: an experience in 242 patients. *Chest* 2003;123(2):604-607.
36. Herth FJ, Eberhardt R, Vilmann P, et al.: Real-time endobronchial ultrasound guided transbronchial needle aspiration for sampling mediastinal lymph nodes. *Thorax* 2006;61(9):795-798.
37. Yasufuku K, Chiyo M, Koh E, et al.: Endobronchial ultrasound guided transbronchial needle aspiration for staging of lung cancer. *Lung Cancer* 2005;50(3):347-354.
38. Harrow EM, Oldenburg FA, Smith AM: Transbronchial needle aspiration to clinical practice. *Thorax* 1985;40:756-759.
39. Baker JJ, Solanki PH, Schenk DA, et al.: Transbronchial fine needle aspiration of the mediastinum: Importance of lymphocytes as an indicator of specimen adequacy. *Acta Cytol* 1990;3:517-523.
40. Rosenthal DL, Wallace JM: Fine needle aspiration of pulmonary lesions via fiberoptic bronchoscopy. *Acta Cytol* 1984;28:203-210.
41. Bhat N, Bhagat P, Pearlman ES, et al.: Transbronchial needle aspiration biopsy in the diagnosis of pulmonary neoplasms. *Diagn Cytopathol* 1990;6:14-17.
42. Zakowski MF, Gatscha RM, Zaman MB: Negative predictive value of pulmonary fine needle aspiration cytology. *Acta Cytol* 1992;6:283-286.
43. Wagner ED, Ramzy I, Greenberg SD, Gonzalez JM: Transbronchial fine-needle aspiration: Reliability and limitations. *Am J Clin Pathol* 1989;92:36-41.
44. Rintoul RC, Skwarski KM, Murchison JT, et al.: Endobronchial and endoscopic ultrasound-guided real-time fine-needle aspiration for mediastinal staging. *Eur Respir J* 2005;25(3):416-421.
45. Singh P, Camazine B, Jadhav Y, et al.: Endoscopic ultrasound as a first test for diagnosis and staging of lung cancer: A prospective study. *Am J Respir Crit Care Med* 2007;175(4):345-354.
46. Eloubeidi MA, Tamhane A, Chen VK, Cerfolio RJ: Endoscopic ultrasound-guided fine-needle aspiration in patients with non-small cell lung cancer and prior negative mediastinoscopy. *Ann Thorac Surg* 2005;80(4):1231-1239.
47. Herth F, Becker HD, Ernst A: Conventional vs endobronchial ultrasound-guided transbronchial needle aspiration: a randomized trial. *Chest* 2004;125(1):322-325.
48. Emery SC, Savides TJ, Behling CA: Utility of immediate evaluation of endoscopic ultrasound-guided transesophageal fine needle aspiration of mediastinal lymph nodes. *Acta Cytol* 2004;48(5):630-634.
49. Fernandez-Esparrach G, Gines A, Belda J, et al.: Transesophageal ultrasound-guided fine needle aspiration improves mediastinal staging in patients with non-small cell lung cancer and normal mediastinum on computed tomography. *Lung Cancer* 2006;54(1):35-40.
50. Vilmann P, Krasnik M, Larsen SS, et al.: Transesophageal endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) biopsy: A combined approach in the evaluation of mediastinal lesions. *Endoscopy* 2005;37(9):833-839.
51. Westcott JL, Rao N, Colley DP: Transthoracic needle biopsy of small pulmonary nodules. *Radiology* 1997;202:97-103.
52. Agarwal PK, Husain N, Singh BN: Cytologic findings in aspirated hydatid fluid. *Acta Cytol* 1989;33:652-654.
53. Austin JHM, Cohen MB: Value of having a cytopathologist present during percutaneous fine-needle aspiration biopsy of lung: Report of 55 cancer patients and metaanalysis of the literature. *Am J Roent* 1993;160:175-177.
54. Sacchini V, Galimberti V, Marchini S, Luini A: Percutaneous transthoracic needle aspiration biopsy: A case report of implantation metastasis. *Eur J Surg Oncol* 1989;15:179-183.
55. Zarbo RJ, Fenoglio-Preiser CM: Interinstitutional database for comparison of performance in lung fine-needle aspiration cytology: A College of American Pathologists Q-probe study of 5264 cases with histologic correlation. *Arch Pathol Lab Med* 1992;116:463-470.

56. Yazdi HM, MacDonald LL, Hickey NM: Thoracic fine needle aspiration biopsy versus fine needle cutting biopsy: A comparative study of 40 patients. *Acta Cytol* 1988;32:635-640.
57. Arslan S, Yilmaz A, Bayramgurler B, et al.: CT-guided transthoracic fine needle aspiration of pulmonary lesions: accuracy and complications in 294 patients. *Med Sci Monit* 2002;8(7):CR493-497.
58. Di Donna A, Bazzocchi M, Dolcet F, Springolo E: [CT-guided transthoracic needle aspiration of solitary lung lesions. Personal experience in 118 cases]. *Radiol Med (Torino)* 1995;89(3):287-294.
59. Hayes MM, Zhang DY, Brown W: Transthoracic fine-needle aspiration biopsy cytology of pulmonary neoplasms. *Diagn Cytopathol* 1994;10(4):315-319.
60. Arakawa H, Nakajima Y, Kurihara Y, et al.: CT-guided transthoracic needle biopsy: A comparison between automated biopsy gun and fine needle aspiration. *Clin Radiol* 1996;51(7):503-506.
61. Diacon AH, Theron J, Schubert P, et al.: Ultrasound-assisted transthoracic biopsy: Fine-needle aspiration or cutting-needle biopsy? *Eur Respir J* 2007;29(2):357-362.
62. Greif J, Marmur S, Schwarz Y, et al.: Percutaneous core cutting needle biopsy compared with fine-needle aspiration in the diagnosis of peripheral lung malignant lesions: Results in 156 patients. *Cancer* 1998;84(3):144-147.
63. Cristallini EG, Ascani S, Farabi R, et al.: Fine needle aspiration biopsy in the diagnosis of intrathoracic masses. *Acta Cytol* 1992;36:416-422.
64. Castella X, Valles J, Cabezu MA, et al.: Fat embolism syndrome and pulmonary microvascular cytology. *Chest* 1992;101:1710-1711.
65. Abati A, Landucci D, Danner RL, Solomon D: Diagnosis of pulmonary microvascular metastases by cytologic evaluation of pulmonary artery catheter-derived blood specimens. *Hum Pathol* 1994;25:257-262.
66. Masson RG, Ruggieri J: Pulmonary microvascular cytology. A new diagnostic application of the pulmonary artery catheter. *Chest* 1985;88:908-914.
67. Dhooge I, Joos G, Van Cauwenberge P: Value of rigid bronchoscopy and cytodiagnosis of bronchial washings in detecting bronchial carcinoma in the presence of a carcinoma of the upper aerodigestive tract. *J Otolaryngol* 1996;25:219-222.
68. Roggli VL, Piantadosi CA, Bell DY: Asbestos bodies in bronchoalveolar lavage fluid: A study of 20 asbestos-exposed individuals and comparison to patients with other chronic interstitial lung diseases. *Acta Cytol* 1986;30:470-476.
69. Strickler JG, Manivel JC, Copenhaver CM, Kubic VL: Comparison of in situ hybridization and immunohistochemistry for detection of cytomegalovirus and herpes simplex virus. *Hum Pathol* 1990;21(4):443-448.
70. Weiss RL, Snow GW, Schumann GB, Hammond ME: Diagnosis of cytomegalovirus pneumonitis on bronchoalveolar lavage fluid: Comparison of cytology, immunofluorescence, and in situ hybridization with viral isolation. *Diagn Cytopathol* 1991;7:243-247.
71. Weinberg A, Hodges TN, Li S, et al.: Comparison of PCR, antigenemia assay, and rapid blood culture for detection and prevention of cytomegalovirus disease after lung transplantation. *J Clin Microbiol* 2000;38(2):768-772.
72. Harboldt SL, Dugan JM, Tronic BS: Cytologic diagnosis of measles pneumonia in a bronchoalveolar lavage specimen. A case report. *Acta Cytol* 1994;38:403-406.
73. Pierce CH, Knox AW: Ciliocytophthoria in sputum from patients with adenovirus infections. *Proc Soc Exp Biol Med* 1960;104:492-495.
74. Osumi M, Toyoda T, Kawashiro T, Aoyagi T: [Evaluation of a new detection method of Mycobacterium tuberculosis in clinical specimens by amplification of ribosomal RNA and its clinical application]. *Kekkaku* 1996;71(10):573-585.
75. Qadri SM, Ali MA: Sensitivity of fine-needle aspiration biopsy in the detection of mycobacterial infections. *Diagn Cytopathol* 1991;7:142-146.
76. Das DK, Bhambhani S, Pant JN, et al.: Superficial and deep-seated tuberculosis lesions: Fine-needle aspiration cytology diagnosis of 547 cases. *Diagn Cytopathol* 1992;8:211-215.
77. DiTomasso JB, Ampel NM, Sobonya RE, Bloom JW: Bronchoscopic diagnosis of pulmonary coccidioidomycosis. Comparison of cytology, culture, and transbronchial biopsy. *Diagn Microbiol Infect Dis* 1994;18:83-87.
78. Raab SS, Silverman JF, Zimmerman KG: Fine-needle aspiration biopsy of pulmonary coccidioidomycosis: Spectrum of cytologic findings in 73 patients. *Am J Clin Pathol* 1993;99:582-587.
79. Iwama de Mattos MC, de Oliveira ML: Respiratory cytopathology: Paracoccidioidomycosis associated either with tuberculosis or bronchogenic carcinoma. *Diagn Cytopathol* 1992;8:198-199.
80. Iwama de Mattos MC, de Oliveira ML: Pseudoepitheliomatous proliferation, a pitfall in sputum cytology. *Diagn Cytopathol* 1991;7:656-657.
81. Iwama de Mattos MC, Mendes RP, Marcondes-Machado J, et al.: Sputum cytology in the diagnosis of pulmonary paracoccidioidomycosis. *Mycopathologica* 1991;114:187-191.
82. Farley ML, Fagan MF, Mabry LC, Wallace RJ, Jr: Presentation of *Sporothrix schenckii* in pulmonary cytology specimens. *Acta Cytol* 1991;35:389-395.
83. Ness MJ, Rennard SI, Vaughn WP, et al.: Detection of Candida antigen in bronchoalveolar lavage fluid. *Acta Cytol* 1988;32:347-352.
84. Edman JC, Kovacs JA, Masur H, et al.: Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. *Nature* 1988;334:519-522.
85. Ypma-Wong MF, Fonzi WA, Sypherd PS: Fungus-specific translation elongation factor 3 gene present in *Pneumocystis carinii*. *Infect Immun* 1992;60:4140-4145.
86. Hartmann B, Koss M, Hui A, et al.: *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome (AIDS): Diagnosis with bronchial brushings, biopsy, and bronchoalveolar lavage. *Chest* 1985;87:603-607.
87. Maiese SC, Naryshkin S: Diagnosis of *Pneumocystis carinii* by cytologic examination of Papanicolaou-stained sputum specimens. *Diagn Cytopathol* 1991;7:111-112.
88. Naryshkin S, Daniels J, Freno E, Cunningham L: Cytology of treated and minimal *Pneumocystis carinii* pneumonia and a pitfall of the Grocott methenamine silver stain. *Diagn Cytopathol* 1991;7:41-47.
89. Kovacs JA, Ng VL, Masur H, et al.: Diagnosis of *Pneumocystis carinii* pneumonia: improved detection in sputum with use of monoclonal antibodies. *N Engl J Med* 1988;318:589-593.
90. Midgley J, Parsons PA, Shanson DC, et al.: Monoclonal immunofluorescence compared with silver stain for investigating *Pneumocystis carinii* pneumonia. *J Clin Pathol* 1991;44:75-76.
91. Hawkins AG, Hsiu JG, Smith RM3rd, et al.: Pulmonary dirofilariasis diagnosed by fine needle aspiration biopsy: A case report. *Acta Cytol* 1985;29:19-22.
92. Ingram EA, Helikson MA: Echinococcosis (hydatid disease) in Missouri: Diagnosis by fine-needle aspiration of a lung cyst. *Diagn Cytopathol* 1991;7:527-531.
93. Aisner SC, Gupta PK, Frost JK: Sputum cytology in pulmonary sarcoidosis. *Acta Cytol* 1977;21:394-398.
94. Travis WD, Colby TV, Koss MN, et al.: Atlas of Nontumor Pathology: Non-neoplastic Disorders of the Lower Respiratory

- Tract. Washington, DC, American Registry of Pathology and the Armed Forces Institute of Pathology, 2002.
95. Kitamura T, Tanaka N, Watanabe J, et al: Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1999;190(6):875-880.
 96. Uchida K, Beck DC, Yamamoto T, et al: GM-CSF autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. *N Engl J Med* 2007;356(6):567-579.
 97. Beskow CO, Drachenberg CB, Bourquin PM, et al: Diffuse alveolar damage. Morphologic features in bronchoalveolar lavage fluid. *Acta Cytol* 2000;44:640-646.
 98. Hyakudo T, Yoshii C, Nikaido Y, et al: A case of bronchiolitis obliterans organizing pneumonia: Diagnostic utility of bronchoalveolar lavage and CT scan. *Nihon Kyobu Shikkan Gakkai Zasshi* 1994;32:768-773.
 99. Majori M, Poletti V, Curti A, et al: Bronchoalveolar lavage in bronchiolitis obliterans organizing pneumonia primed by radiation therapy to the breast. *J Allergy Clin Immunol* 2000;105:239-244.
 100. Poletti V, Cazzato S, Minicuci N, et al: The diagnostic value of bronchoalveolar lavage and transbronchial lung biopsy in cryptogenic organizing pneumonia. *Eur Respir J* 1996;9:2513-2516.
 101. Tiroke AH, Bewig B, Haverich A: Bronchoalveolar lavage in lung transplantation. State of the art. *Clin Transplant* 1999;13:131-157.
 102. Walts AE, Marchevsky AM, Morgan M: Pulmonary cytology in lung transplant recipients: Recent trends in laboratory utilization. *Diagn Cytopathol* 1991;7:353-358.
 103. Minoletti F, Sozzi G, Calderone C, et al: Variant translocation t(6;10)(p21;q22) in pulmonary chondroid hamartoma. *Genes Chromosomes Cancer* 1996;15:246-248.
 104. Xiao S, Lux ML, Reeves R, et al: HMG(Y) activation by chromosome 6p21 rearrangements in multilineage mesenchymal cells from pulmonary hamartoma. *Am J Pathol* 1997;150:901-910.
 105. Dunbar F, Leiman G: The aspiration cytology of pulmonary hamartomas. *Diagn Cytopathol* 1989;5:174-180.
 106. Hughes JH, Young NA, Wilbur DC, et al: Fine-needle aspiration of pulmonary hamartoma: A common source of false-positive diagnoses in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology. *Arch Pathol Lab Med* 2005;129(1):19-22.
 107. Meis-Kindblom JM, Kjellstrom C, Kindblom LG: Inflammatory fibrosarcoma: Update, reappraisal, and perspective on its place in the spectrum of inflammatory myofibroblastic tumors. *Semin Diagn Pathol* 1998;15:133-143.
 108. Lawrence B, Perez-Atayde A, Hibbard MK, et al: TPM3-ALK and TPM4-ALK oncogenes in inflammatory myofibroblastic tumors. *Am J Pathol* 2000;157:377-384.
 109. Bridge JA, Kanamori M, Ma Z, et al: Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor. *Am J Pathol* 2001;159(2):411-415.
 110. Debiec-Rychter M, Marynen P, Hagemeijer A, Pauwels P: ALK-AT1C fusion in urinary bladder inflammatory myofibroblastic tumor. *Genes Chromosomes Cancer* 2003;38(2):187-190.
 111. Usuda K, Saito Y, Imai T, et al: Inflammatory pseudotumor of the lung diagnosed as granulomatous lesion by preoperative brushing cytology: A case report. *Acta Cytol* 1990;34:685-689.
 112. Füzesi L, Höer PW, Schmidt W: Exfoliative cytology of multiple endobronchial granular cell tumor. *Acta Cytol* 1989;33:516-518.
 113. Mermolja M, Rott T: Cytology of endobronchial granular cell tumor. *Diagn Cytopathol* 1991;7:524-526.
 114. Coissard CJ, Besson G, Polette MC, et al: Prevalence of human papillomaviruses in lung carcinomas: a study of 218 cases. *Mod Pathol* 2005;18(12):1606-1609.
 115. Nadal A, Campo E, Pinto J, et al: p53 expression in normal, dysplastic, and neoplastic laryngeal epithelium. Absence of a correlation with prognostic factors. *J Pathol* 1995;175:181-188.
 116. Rusch V, Klimstra D, Linkov I, Dmitrovsky E: Aberrant expression of p53 or the epidermal growth factor receptor is frequent in early bronchial neoplasia and coexpression precedes squamous cell carcinoma development. *Cancer Res* 1995;55:1365-1372.
 117. Vine MF, Schoenbach VJ, Hulka BS, et al: Atypical metaplasia and incidence of bronchogenic carcinoma. *Am J Epidemiol* 1990;131:781-793.
 118. Onuki N, Wistuba II, Travis WD, et al: Genetic changes in the spectrum of neuroendocrine lung tumors. *Cancer* 1999;85:600-607.
 119. Lam S, Becker HD: Future diagnostic procedures. *Chest Surg Clin N Am* 1996;6:363-380.
 120. Hammond WG, Teplitz RL, Benfield JR: Variable regression of experimental bronchial preneoplasia during carcinogenesis. *J Thorac Cardiovasc Surg* 1991;101:800-806.
 121. Jemal A, Siegel R, Ward E, et al: Cancer statistics, 2007. *CA Cancer J Clin* 2007;57(1):43-66.
 122. Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC (eds): World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. Lyon, IARC Press, 2004.
 123. Ahrendt SA, Chow JT, Xu LH, et al: Molecular detection of tumor cells in bronchoalveolar lavage fluid from patients with early stage lung cancer. *J Natl Cancer Inst* 1999;91:332-339.
 124. Somers VA, Pietersen AM, Theunissen PH, Thunnissen FB: Detection of K-ras point mutations in sputum from patients with adenocarcinoma of the lung by point-EXACCT. *J Clin Oncol* 1998;16:3061-3068.
 125. Hussain SP, Harris CC: p53 mutation spectrum and load: The generation of hypotheses linking the exposure of endogenous or exogenous carcinogens to human cancer. *Mutat Res* 1999;428:23-32.
 126. Fontana RS, Sanderson DR, Woolner LB, et al: Lung cancer screening: The Mayo program. *J Occup Med* 1986;28:746-750.
 127. McGee JMC: Screening for lung cancer. *Semin Surg Oncol* 1989;5:179-185.
 128. Swensen SJ, Jett JR, Sloan JA, et al: Screening for lung cancer with low-dose spiral computed tomography. *Am J Respir Crit Care Med* 2002;165(4):508-513.
 129. Diederich S, Thomas M, Semik M, et al: Screening for early lung cancer with low-dose spiral computed tomography: Results of annual follow-up examinations in asymptomatic smokers. *Eur Radiol* 2004;14(4):691-702.
 130. Patz EF Jr, Swensen SJ, Herndon JE 2nd. Estimate of lung cancer mortality from low-dose spiral computed tomography screening trials: implications for current mass screening recommendations. *J Clin Oncol* 2004;22(11):2202-2206.
 131. Sone S, Li F, Yang ZG, et al: Results of three-year mass screening programme for lung cancer using mobile low-dose spiral computed tomography scanner. *Br J Cancer* 2001;84(1):25-32.
 132. Sone S, Nakayama T, Honda T, et al: Long-term follow-up study of a population-based 1996-1998 mass screening programme for lung cancer using mobile low-dose spiral computed tomography. *Lung Cancer* 2007;58(3):329-341.
 133. Jett JR: Limitations of screening for lung cancer with low-dose spiral computed tomography. *Clin Cancer Res* 2005;11(13 Pt 2):4988s-4992s.
 134. Manser R, Dalton A, Carter R, et al: Cost-effectiveness analysis of screening for lung cancer with low dose spiral CT (computed tomography) in the Australian setting. *Lung Cancer* 2005;48(2):171-185.
 135. Pastorino U: Early detection of lung cancer. *Respiration* 2006;73(1):5-13.

136. Li R, Todd NW, Qiu Q, et al.: Genetic deletions in sputum as diagnostic markers for early detection of stage I non-small cell lung cancer. *Clin Cancer Res* 2007;13(2 Pt 1):482-487.
137. Kersting M, Friedl C, Kraus A, et al.: Differential frequencies of p16(INK4a) promoter hypermethylation, p53 mutation, and K-ras mutation in exfoliative material mark the development of lung cancer in symptomatic chronic smokers. *J Clin Oncol* 2000;18(18):3221-3229.
138. Proctor L, Folpe AL, Esper A, et al.: Well-differentiated fetal adenocarcinoma of the lung: Cytomorphologic features on fine-needle aspiration with emphasis on use of beta-catenin as a useful diagnostic marker. *Diagn Cytopathol* 2007;35(1):39-42.
139. Paez JG, Janne PA, Lee JC, et al.: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304(5676):1497-1500.
140. Marchetti A, Martella C, Felicioni L, et al.: EGFR mutations in non-small-cell lung cancer: Analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 2005;23(4):857-865.
141. Haneda H, Sasaki H, Lindeman N, et al.: A correlation between EGFR gene mutation status and bronchioloalveolar carcinoma features in Japanese patients with adenocarcinoma. *Jpn J Clin Oncol* 2006;36(2):69-75.
142. Chen YR, Fu YN, Lin CH, et al.: Distinctive activation patterns in constitutively active and gefitinib-sensitive EGFR mutants. *Oncogene* 2006;25(8):1205-1215.
143. Okabe T, Okamoto I, Tamura K, et al.: Differential constitutive activation of the epidermal growth factor receptor in non-small cell lung cancer cells bearing EGFR gene mutation and amplification. *Cancer Res* 2007;67(5):2046-2053.
144. Greulich H, Chen TH, Feng W, et al.: Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. *PLoS Med* 2005;2(11):e313.
145. Lynch TJ, Bell DW, Sordella R, et al.: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350(21):2129-2139.
146. Inoue A, Suzuki T, Fukuhara T, et al.: Prospective phase II study of gefitinib for chemotherapy-naïve patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. *J Clin Oncol* 2006;24(21):3340-3346.
147. Jackman DM, Yeap BY, Lindeman NI, et al.: Phase II clinical trial of chemotherapy-naïve patients > or = 70 years of age treated with erlotinib for advanced non-small-cell lung cancer. *J Clin Oncol* 2007;25(7):760-766.
148. Engelman JA, Zejnullahu K, Mitsudomi T, et al.: MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007;316(5827):1039-1043.
149. Riely GJ, Kris MG, Zhao B, et al.: Prospective assessment of discontinuation and reinitiation of erlotinib or gefitinib in patients with acquired resistance to erlotinib or gefitinib followed by the addition of everolimus. *Clin Cancer Res* 2007;13(17):5150-5155.
150. Smouse J, Cibas E, Janne P, et al.: Cytology specimens compare favorably with surgical specimens for EGFR mutation detection in patients with NSCLC. *Mod Pathol* 2007;20 (Suppl 2):85A.
151. Horike A, Kimura H, Nishio K, et al.: Detection of epidermal growth factor receptor mutation in transbronchial needle aspirates of non-small cell lung cancer. *Chest* 2007;131(6):1628-1634.
152. Chen KT: Psammoma bodies in fine-needle aspiration cytology of papillary adenocarcinoma of the lung. *Diagn Cytopathol* 1990;6:271-274.
153. Silverman JE, Finley JL, Park HK, et al.: Psammoma bodies and optically clear nuclei in bronchioloalveolar cell carcinoma: Diagnosis by fine needle aspiration biopsy with histologic and ultrastructural confirmation. *Diagn Cytopathol* 1985;1:205-215.
154. Ohori NP, Santa Maria EL: Cytopathologic diagnosis of bronchioloalveolar carcinoma: does it correlate with the 1999 World Health Organization definition? *Am J Clin Pathol* 2004;122(1):44-50.
155. Mhoyan A, Weidner N, Shabaik A: Epithelioid hemangioendothelioma of the lung diagnosed by transesophageal endoscopic ultrasound-guided fine needle aspiration: A case report. *Acta Cytol* 2004;48(4):555-559.
156. Travis WD, Linnoila RI, Tsokos MG, et al.: Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma: An ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. *Am J Surg Pathol* 1991;15:529-553.
157. Hammond ME, Sause WT: Large cell neuroendocrine tumors of the lung: Clinical significance and histopathologic definition. *Cancer* 1985;56:1624-1629.
158. Iwasaki Y: Large cell carcinoma. *Nippon Rinsho* 2000;58:1127-1131.
159. Koss MN, Hochholzer L, O'Leary T: Pulmonary blastomas. *Cancer* 1991;67:2368-2381.
160. Holst VA, Finkelstein S, Colby TV, et al.: p53 and K-ras mutational genotyping in pulmonary carcinosarcoma, spindle cell carcinoma, and pulmonary blastoma: Implications for histogenesis. *Am J Surg Pathol* 1997;21:801-811.
161. Cosgrove MM, Chandrasoma PT, Martin SE: Diagnosis of pulmonary blastoma by fine-needle aspiration biopsy: cytologic and immunocytochemical findings. *Diagn Cytopathol* 1991;7:83-87.
162. Warren WM, Faber LP, Gould VE: Neuroendocrine neoplasms of the lung: a clinicopathologic update. *J Thorac Cardiovasc Surg* 1989;98:321-332.
163. Fekete PS, Cohen C, DeRose PB: Pulmonary spindle cell carcinoid: Needle aspiration biopsy, histologic and immunohistochemical findings. *Acta Cytol* 1990;34:50-56.
164. Jordan AG, Predmore L, Sullivan MM, Memoli VA: The cytodagnosis of well-differentiated neuroendocrine carcinoma: A distinct clinicopathologic entity. *Acta Cytol* 1987;31:464-470.
165. Travis WD, Rush W, Flieder DB, et al.: Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. *Am J Surg Pathol* 1998;22(8):934-944.
166. Renshaw AA, Voytek TM, Haja J, Wilbur DC: Distinguishing small cell carcinoma from non-small cell carcinoma of the lung: correlating cytologic features and performance in the College of American Pathologists Non-Gynecologic Cytology Program. *Arch Pathol Lab Med* 2005;129(5):619-623.
167. Kalhor N, Zander DS, Liu J: TTF-1 and p63 for distinguishing pulmonary small-cell carcinoma from poorly differentiated squamous cell carcinoma in previously pap-stained cytologic material. *Mod Pathol* 2006;19(8):1117-1123.
168. Wu M, Wang B, Gil J, et al.: p63 and TTF-1 immunostaining. A useful marker panel for distinguishing small cell carcinoma of lung from poorly differentiated squamous cell carcinoma of lung. *Am J Clin Pathol* 2003;119(5):696-702.
169. Dalton ML, Gatling RR: Peripheral adenoid cystic carcinoma of the lung. *South Med J* 1990;83:577-579.
170. Tonon G, Modi S, Wu L, et al.: t(11;19)(q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. *Nat Genet* 2003;33(2):208-213.
171. Lantuejoul S, Isaac S, Pinel N, et al.: Clear cell tumor of the lung: An immunohistochemical and ultrastructural study supporting a pericytic differentiation. *Mod Pathol* 1997;10:1001-1008.
172. Policarpio-Nicolas ML, Covell J, Bregman S, Atkins K: Fine needle aspiration cytology of clear cell "sugar" tumor

- (PEComa) of the lung: Report of a case. *Diagn Cytopathol* 2008;36(2):89–93.
173. Husain M, Nguyen GK: Cytopathology of granular-cell tumor of the lung. *Diagn Cytopathol* 2000;23(4):294–295.
 174. Gal AA, Koss MN, Hochholzer L, Chejfec G: An immunohistochemical study of benign clear cell (“sugar”) tumor of the lung. *Arch Pathol Lab Med* 1991;115:1034–1038.
 175. Fleming WH, Jove DF: Primary leiomyosarcoma of the lung with positive sputum cytology. *Acta Cytol* 1975;19:14–20.
 176. Moran CA, Suster S: Tumors of the lung and pleura. In Fletcher C (ed): *Diagnostic Histopathology of Tumors*, 3rd ed. New York, Churchill Livingstone, 2007, pp. 181–214.
 177. Stanfield BL, Powers CN, Desch CE, et al: Fine-needle aspiration cytology of an unusual primary lung tumor, chondrosarcoma: Case report. *Diagn Cytopathol* 1991;7:423–426.
 178. Nguyen GK: Exfoliative cytology of angiosarcoma of the pulmonary artery. *Acta Cytol* 1985;29:624–627.
 179. Crosby JH, Hoeg K, Hager B: Transthoracic fine needle aspiration of primary and metastatic sarcomas. *Diagn Cytopathol* 1985;1:221–227.
 180. Kim K, Naylor B, Han IH: Fine needle aspiration cytology of sarcomas metastatic to the lung. *Acta Cytol* 1986;30(6):688–694.
 181. Flieder DB, Yousem SA: Pulmonary lymphomas and lymphoid hyperplasias. In Knowles D(ed) *Neoplastic Hematopathology*. Baltimore, Lippincott, Williams & Wilkins, 2001, pp. 1263–1301.
 182. Habermann TM, Ryu JH, Inwards DJ, Kurtin PJ: Primary pulmonary lymphoma. *Semin Oncol* 1999;26:307–315.
 183. Pisani RJ, DeRemee RA: Clinical implications of the histopathologic diagnosis of pulmonary lymphomatoid granulomatosis. *Mayo Clin Proc* 1990;65:151–163.
 184. Myers JL, Kurtin PJ, Katzenstein AL, et al: Lymphomatoid granulomatosis. Evidence of immunophenotypic diversity and relationship to Epstein-Barr virus infection. *Am J Surg Pathol* 1995;19:1300–1312.
 185. Zaer FS, Braylan RC, Zander DS, et al: Multiparametric flow cytometry in the diagnosis and characterization of low-grade pulmonary mucosa associated lymphoid tissue lymphomas. *Mod Pathol* 1998;11:525–532.
 186. Sprague RI, deBlois GG: Small lymphocytic pulmonary lymphoma: diagnosis by transthoracic fine needle aspiration. *Chest* 1989;96:929–930.
 187. Reale FR, Variakojis D, Compton J, Bibbo M: Cytodiagnosis of Hodgkin's disease in sputum specimens. *Acta Cytol* 1983;27:258–261.
 188. Rossi GA, Balbi B, Risso M, et al: Acute myelomonocytic leukemia: Demonstration of pulmonary involvement by bronchoalveolar lavage. *Chest* 1985;87:259–260.
 189. Riazmontazer N, Bedayat G: Cytology of plasma cell myeloma in bronchial washing. *Acta Cytol* 1989;33:519–522.
 190. Rosen SE, Vonderheid EC, Koprowska I: Mycosis fungoides with pulmonary involvement: cytopathologic findings. *Acta Cytol* 1984;28:51–57.
 191. Rosenblatt MB, Lisa JR, Trinidad S: Pitfalls in the clinical and histologic diagnosis of bronchogenic carcinoma. *Dis Chest* 1966;49:396–404.
 192. Kern WH, Schweizer CW: Sputum cytology of metastatic carcinoma of the lung. *Acta Cytol* 1976;20:514–520.
 193. Johnston WW: Cytologic correlations. In Dail K, Hammer S(eds): *Pulmonary Pathology*. New York, Springer-Verlag, 1987, pp. 1058.
 194. Johnston WW: Percutaneous fine needle aspiration biopsy of the lung: a study of 1,015 patients. *Acta Cytol* 1984;28:218–224.
 195. Zaman MB, Hajdu SI, Melamed MR, Watson RC: Transthoracic aspiration cytology of pulmonary lesions. *Semin Diagn Pathol* 1986;3:176–187.
 196. Holzinger A, Dingle S, Bejarano PA, et al: Monoclonal antibody to thyroid transcription factor-1: Production, characterization, and usefulness in tumor diagnosis. *Hybridoma* 1996;15:49–53.
 197. Weir BA, Woo MS, Getz Get al.: Characterizing the cancer genome in lung adenocarcinoma. *Nature* 2007; 450(7171):893–898.

Urine and Bladder Washings

ANDREW A. RENSHAW

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DIAGNOSING DIFFICULT OR BORDERLINE SPECIMENS: COMMON PATTERNS

ANCILLARY TECHNIQUES

SUMMARY

Urine cytology was popularized by George Papanicolaou in the 1940s as a way to detect and follow patients with bladder cancer.¹ By the 1960s, the cytologic, histologic, and clinical features of high-grade urothelial carcinoma (UC) were well established.² Despite many attempts to develop another test with greater sensitivity and specificity, cytology remains one of the best (i.e., inexpensive, quick, and reliable) ways to diagnose a variety of bladder lesions, most importantly high-grade UC.

Clinical indications for urine cytology:

- hematuria
- follow-up of patients treated for UC
- high risk of bladder cancer

The most common indication for urinary cytology is hematuria.^{3,4} The yield, however, is low; hematuria is caused by a malignancy in only 5% to 10% of patients.⁵⁻⁷ Another indication is surveillance for recurrent UC because patients with a previously diagnosed and treated UC are at risk for recurrence or a de novo primary elsewhere in the urinary tract.² Urine cytology is not used for screening asymptomatic individuals because the benefits are outweighed by the considerable cost.⁸⁻¹⁰ It is used, however, when an individual has risk factors for bladder cancer, such as an occupational exposure to aniline dyes,¹¹ the aromatic amines used in the petrochemical industry,¹² or cyclophosphamide treatment for diseases like multiple sclerosis.¹³

SPECIMEN COLLECTION

There are six types of urinary specimens, each with its relative advantages (Table 3.1).

Voided Urine

Voided urine should be obtained 3 to 4 hours after the patient has last urinated.¹⁴⁻¹⁶ First morning voided urine specimens should be avoided because stagnant cells in the low acid-base balance (pH) and hypertonic environment undergo degenerative changes, making cytologic assessment difficult. The minimum amount of urine necessary to ensure adequate cellularity is unknown, but it may be as high as 25 to 100 mL.¹⁷

In women, voided urine can be contaminated by vaginal cells, but in most instances this does not compromise a diagnosis. Still, to help ensure the adequacy of the sample, a midstream ("clean catch") specimen is recommended.

Catheterized Urine

Specimens obtained by catheterization have disadvantages for both the patient and the cytologist. First, catheterization carries a risk of urinary tract infection (UTI). Second, urine collected from an indwelling catheter is often a pooled specimen that has been at room temperature for many hours, and cellular degeneration can be pronounced. Third, the tip of the catheter often scrapes off benign urothelial cell clusters, which mimic the appearance of a low-grade papillary neoplasm.

Bladder Washings

Bladder washings are obtained through a catheter by irrigating the bladder with 5 to 10 pulses of 50 mL of sterile

normal saline, which produce a cellular suspension of freshly exfoliated epithelial cells. This specimen is collected before any biopsy sampling. The chief advantages of bladder washings over voided urine are better cellular preservation, greater cellularity, and less chance of contamination by background debris.

Upper Tract Washings and Brushings

When an upper urinary tract malignancy is suspected, directed washings, brushings, or biopsies of a ureter or renal pelvis lesion can be performed. Although brushings obtained by direct visualization using an endoscope were introduced in 1973, they are rarely obtained. Nevertheless, the sensitivity and specificity of brushings compares favorably with other (voided, catheterized, and irrigation) cytologic methods.¹⁸

The most common upper tract specimen is the directed washing. (With improvements in technology, however, more upper tract biopsies are now being obtained.¹⁹) Directed washing specimens are particularly challenging, for urologists and cytologists.¹⁹⁻²⁵ Urologists often cannot visualize lesions in the upper tract as well as those in the bladder, hence they rely on cytology here even more than for lesions in the bladder. (Although they may try to obtain a biopsy, often these biopsies are small and crushed.) The stakes are high, because the operation of choice for a tumor in the upper tract is removal of the kidney or ureter. In most cases, the significant imaging finding is a filling defect, and the differential diagnosis is a tumor or a stone. Unfortunately, benign cytologic atypia produced by some stones can mimic the cytologic features of urothelial neoplasms. Finally, normal specimens from the upper tract often show diffuse nuclear enlargement, an elevated nuclear-to-cytoplasmic ratio, and high

TABLE 3.1 RELATIVE ADVANTAGES AND DISADVANTAGES OF URINE SPECIMEN TYPES

Specimen Type	Advantages	Disadvantages
Voided urine	Noninvasive No instrumentation artifact	Low cellularity Vaginal contamination Poor preservation
Catheterized	High cellularity	Invasive Instrumentation artifact Poor preservation
Bladder washing	High cellularity Good cell preservation	Invasive Instrumentation artifact
Upper tract washing	High cellularity Good preservation Selective sampling	Invasive Instrumentation artifact
Brush cytology	Selective sampling	Invasive Air drying possible (if direct smear)
Ileal loop	Permits screening for recurrent bladder cancer	Low cellularity Poor preservation

cellularity. These entirely benign changes can suggest a tumor and result in a false-positive diagnosis.²³ For these reasons, it is impossible to accurately diagnose low-grade lesions in upper tract specimens. For high-grade tumors, the sensitivity of ureteral washing cytology and ureteral biopsy for the detection of malignancy is similar and approaches 70% to 80%.¹⁹ With bilateral specimens, one can compare subtle changes between a lesion (on one side) and presumably normal specimen (on the other side). The preparation of a “cell block” (a formalin-fixed, paraffin-embedded sediment of the urine sample) can be particularly useful because small pieces of tumor are often easier to evaluate with this preparation method.^{19,26}

Ileal Conduits

At the time of cystectomy for bladder cancer, a segment of ileum is isolated and reanastomosed to the ureters (or to one ureter if a nephrectomy is also performed) to provide a conduit for urine. Urine samples from these conduits contain a large number of degenerated intestinal epithelial cells. It is important that these specimens be screened for malignant cells because patients with a history of bladder cancer have an increased risk for developing tumors of the ureters and kidneys.²⁷

PROCESSING

Fresh specimens, between 1 and 12 hours old, do not need fixation. If it will take a specimen 12 to 24 hours to reach the laboratory, refrigeration is recommended, and if more than 24 hours, preservation with an equal volume of 50% to 70% ethanol $\pm$ 2% carbowax is advised to avoid degeneration.¹⁶

Slides can be prepared using a variety of concentration techniques, depending on the resources and preferences of the laboratory. These include sedimentation and smearing, membrane filtration, cytocentrifugation, and thinlayer methods. Slides prepared using one of these methods are fixed in ethyl alcohol and stained with the Papanicolaou stain. Urine samples and bladder washings can also be prepared using the cell block technique²⁶; the centrifuged sediment is fixed in formalin, and the slides are stained using hematoxylin and eosin (H & E).

REPORTING TERMINOLOGY AND ADEQUACY CRITERIA

Clinically meaningful adequacy criteria have not been defined. Nevertheless, there are four situations in which the question of adequacy arises.

Patterns of nondiagnostic (unsatisfactory) specimens:

- vaginal cells only
- obscuring inflammation or lubricant
- blood only
- marked degenerative changes

With the exception of ileal pouch specimens, a urine sample must contain at least some urothelial cells to ensure that it is indeed urine. For example, voided urine from women commonly consists almost entirely of squamous cells from the vagina. In some cases, the urothelial cells are obscured by abundant acute inflammation, presumably resulting from an infection or by lubricant jelly. Some specimens consist only of blood. In all three cases, it is reasonable to require the identification of at least one urothelial cell to be sure the specimen is adequate and represents urine.

Finally, degenerated cells are quite common. If the specimen is so degenerated that an interpretation cannot be made, the specimen is inadequate. If only some cells are degenerated, and particularly if the degenerated cells appear atypical, it is advisable to diagnose these specimens as abnormal rather than nondiagnostic.

In most laboratories, the results of urine cytology and bladder washings are reported using simple diagnostic categories such as “negative” (no malignant cells identified), “atypical” (mildly atypical urothelial cells), “suspicious” (abnormal-appearing urothelial cells suspicious for carcinoma), and “positive” (conclusive for malignancy). Because urine cytology is an insensitive test for low-grade urothelial neoplasms, some laboratories prefer the phrase “Negative for a high grade malignancy/carcinoma” rather than “Negative for malignant cells.” For reasons detailed later in the chapter, it is recommended that the “atypical” category be used as sparingly as possible.

ACCURACY

Urine

Urine cytology is, at best, only moderately sensitive in detecting bladder cancer. A representative summary of published studies on the sensitivity of cytology is provided in [Table 3.2](#).²⁸⁻³³ These studies likely overestimate the sensitivity of cytology because virtually all are affected by selection bias; patients with a malignant biopsy are more likely to have had a suspicious or positive cytology. “Positive” results are obtained in 25% to 72% of patients with bladder cancer when all grades and stages of tumors are included in the analysis. Several variables affect the sensitivity of urine cytology. First, sensitivity is higher (37% to 89%)

TABLE 3.2 SENSITIVITY OF URINE AND BLADDER WASHING CYTOLOGY FOR THE DIAGNOSIS OF BLADDER TUMORS

	Number of Specimens Examined Per Patient	Number of Biopsy-Proven Bladder Cancers	Positive Cytology (%)	Suspicious and Positive Cytology (%)
URINE				
Umiker, 1964 ^{a*}	NS	754	72	80
Schoones et al., 1971 ^b	1	114	70	80
Rife et al., 1979 ^c	NS	634	64	76
Kern, 1984 ^d	NS	125	71	89
Koss et al., 1985 ^e	3	181	NS	83
Badalament et al., 1987 ^f	1	228	49	64
Badalament et al., 1987 [‡]	3	149	62	74
Zein, Milad, 1991 ^g	1	362	65	81
Wiener et al., 1993 ^h	NS	84 [‡]	NS	71
Bastacky et al., 1999 ⁱ	variable	180	NS	64
Halling et al., 2000 ^j	1	69	NS	58
Raab et al., 2007 ^k	variable	291	25	37
BLADDER WASHINGS				
Esposti, Zajicek, 1972 ^l	1	274	71	82
Lewis et al., 1976 ^m	1	60	70	80
Loening et al., 1978 ^{n§}	1	262	77	NS
Zein et al., 1984 ^o	1	73	74	89
Badalament et al., 1987 ^f	1	59	66	NS
Curry, Wojcik, 2002 ^p	1	87	NS	53

NS, not specified.

*Review of multiple studies.

[†]Superficial bladder tumors (TIS, TA, T1) only.

[‡]Untreated patients only.

[§]Includes catheterized urine samples.

^aUmiker W: Accuracy of cytologic diagnosis of cancer of the urinary tract. *Acta Cytol* 1964;8:186-193.

^bSchoones R, Gamarra MG, Moore GP: The diagnostic value of urinary cytology in patients with bladder carcinoma. *J Urol* 1971;106:693-696.

^cRife CC, Farrow GM, Utz DC: Urine cytology of transitional cell neoplasms. *Urol Clin N Am* 1979;6:599-612.

^dKern WH: The grade and pathologic stage of bladder cancer. *Cancer* 1984;53:1185-1189.

^eKoss LG, Deitch D, Ramanathan R, Sherman AB: Diagnostic value of cytology of voided urine. *Acta Cytol* 1985;29:810-816.

^fBadalament RA, Hermansen DK, Kimmel M, et al.: The sensitivity of bladder wash flow cytometry, bladder wash cytology, and voided cytology in the detection of bladder carcinoma. *Cancer* 1987;60:1423-1427.

^gZein TA, Milad MF: Urine cytology in bladder tumors. *Int Surg* 1991;76:52-54.

^hWiener HG, Vooijs GP, van't Hof-Grootenboer B: Accuracy of urinary cytology in the diagnosis of primary and recurrent bladder cancer. *Acta Cytol* 1993;37:163-169.

ⁱBastacky S, Ibrahim S, Wilczynski SP, Murphy WM: The accuracy of urinary cytology in daily practice. *Cancer Cytopathol* 1999;87:118-128.

^jHalling KC, King W, Sokolova IA, et al.: A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. *J Urol* 2000;164:1768-1775.

^kRaab SS, Grzybicki DM, Vrbic CM, Geisinger KR: Urine cytology discrepancies: Frequency, causes, and outcomes. *Am J Clin Pathol* 2007;127(6):946-953.

^lEsposti PL, Zajicek J: Grading of transitional cell neoplasms of the urinary bladder from smears of bladder washings. *Acta Cytol* 1972;16:529-537.

^mLewis RW, Jackson AC, Murphy WM, et al.: Cytology in the diagnosis and followup of transitional cell carcinoma of the urothelium: A review with a case series. *J Urol* 1976;116:43-46.

ⁿLoening S, Narayana A, Yoder L, et al.: Longitudinal study of bladder cancer with cytology and biopsy. *Br J Urol* 1978;50:496-501.

^oZein T, Wajsman Z, Englander LS, et al.: Evaluation of bladder washings and urine cytology in the diagnosis of bladder cancer and its correlation with selected biopsies of the bladder mucosa. *J Urol* 1984;132:670-671.

^pCurry JL, Wojcik EM: The effects of the current World Health Organization/International Society of Urologic Pathologists bladder neoplasm classification system on urine cytology results. *Cancer* 2002;96(3):140-145.

when suspicious diagnoses are included with positive diagnoses.^{34,35} Second, the sensitivity increases when more than one specimen is examined; tumor cells may be absent from one urine specimen but present in subsequent specimens.^{34,36,37} For this reason, it has been recommended that at least three specimens per

patient be examined.³⁸ Third, the sensitivity of urine cytology is highly dependent on the grade of the bladder tumor. Low-grade UCs are detected less reliably by cytology, as compared to high-grade UCs.^{34,35,39-41} Finally, the sensitivity of urine cytology is reduced in patients who have been treated with radiation or

chemotherapy.³⁵ Molecular cytogenetic analysis using the fluorescence in situ hybridization (FISH) method shows promise in improving on the sensitivity of cytology.

The moderate sensitivity of cytology is complemented by its high specificity (range of most studies: 95% to 100%).⁴⁰ False-positive results, in other words, are uncommon. Some investigators report finding no false-positive results in their studies^{34,42}; others describe rates ranging from 1.3% to 15%.^{38,43,44} False-positive results occur in patients with bladder stones,⁴⁵ human polyomavirus infection,⁴⁶ and chemotherapy.³⁹ A positive cytologic result in the face of a negative biopsy result does not necessarily mean that the cytologic diagnosis is false. In many cases, a carcinoma that escaped histologic detection is discovered on a subsequent cystoscopic examination. Other sites besides the bladder can be the source of the malignant cells; a primary tumor of the ureters, kidneys, prostate, and other contiguous organs must be considered.^{15,47}

Urine cytology is complemented by cystoscopy. Low-grade tumors missed by cytology are papillary lesions readily visualized with the help of a cystoscope and thus earmarked for biopsy.^{48,49} For high-grade UCs, however, particularly carcinoma in situ (CIS), which is more difficult to detect by cystoscopy, urine cytology provides a high degree of diagnostic accuracy.

Bladder Washings

Bladder washings have the advantage over urine samples in improved cellularity and cell preservation. The sensitivity of a positive bladder washing cytology is slightly higher than that of urine cytology, ranging from 66% to 77% when all grades and stages of bladder tumor are included (see Table 3.2). The superiority of bladder washings over voided urine cytology has been well documented.⁵⁰⁻⁵² This is not to say that voided urine from patients undergoing cystoscopy can be neglected. From 7% to 13% of bladder tumors not detected by bladder washings are discovered in urine samples obtained before cystoscopic examination.^{51,53}

Bladder washing cytology is not without its drawbacks. Most significantly, catheterization is required to obtain the specimen. Bladder washings sample the bladder epithelium only, whereas urine contains cells exfoliated from the ureters and kidneys. In addition, the cellularity of bladder washings depends on the skill of the urologist; if irrigation is not vigorous enough, the specimen can be hypocellular.

Washings of the ureter and pelvis have similar sensitivity (70% to 80% for high-grade lesions) but are particularly prone to false-positive results²³ because of the marked cellularity of these specimens.

NORMAL ELEMENTS

Normal elements:

- urothelial cells
 - intermediate and superficial (umbrella) cells (voided urine)
 - intermediate, superficial, and basal cells (catheterized urine, washings)
- squamous cells
- seminal vesicle epithelial cells (rare)
- degenerated intestinal epithelial cells (ileal conduit specimens)

A normal voided urine specimen is sparsely cellular, but urothelial (or transitional) cells are usually present (Fig. 3.1). They are dispersed as isolated individual cells; tight clusters of urothelial cells are distinctly uncommon in a normal voided urine sample. In voided specimens, most urothelial cells are intermediate in size, with a moderate amount of homogeneously granular or finely vacuolated cytoplasm, round nuclei, and small nucleoli. In some cases they are columnar or spindled; this is a normal finding, although the reason for these shapes is not known (Figs. 3.2 and 3.3). When degenerated, urothelial cells resemble histiocytes, especially because they sometimes contain round, red, or green hyaline cytoplasmic inclusions called Melamed-Wolinska bodies⁵⁴ (see Fig. 3.2). They are seen in almost 50% of urine specimens and are more common in voided as opposed to catheterized samples. The pathogenesis of these bodies is obscure, and they have no diagnostic value in urine, but they are useful in suggesting a urothelial origin for malignant cells in pleural effusions.⁵⁵ Umbrella cells are large and have abundant cytoplasm and large nuclei;

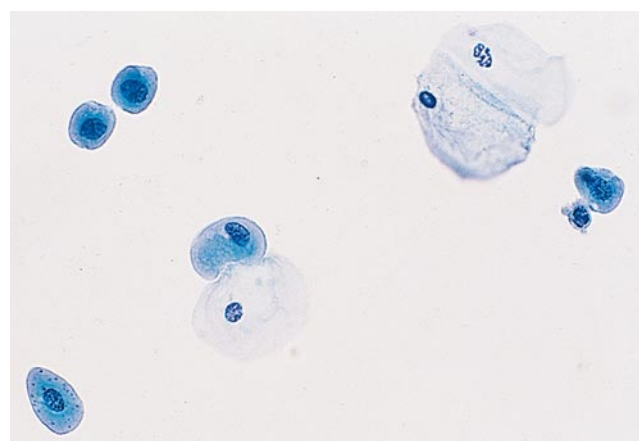


Figure 3.1 Normal voided urine. Most benign voided urine samples show a mixture of urothelial cells and squamous cells. In voided urine, most of the urothelial cells are of “intermediate” type, with an oval or pyramidal shape.

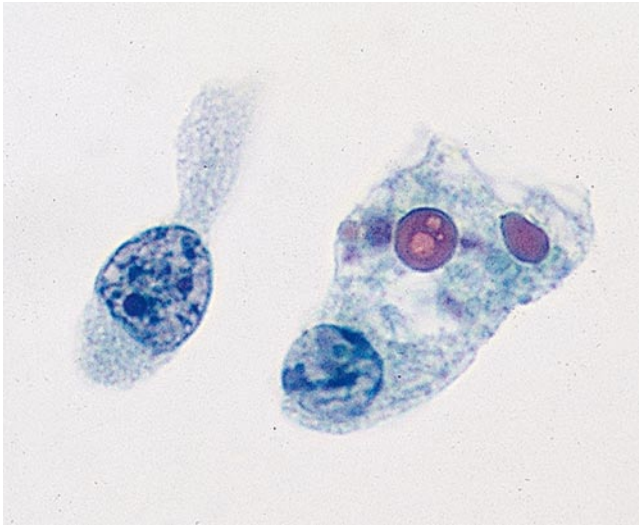


Figure 3.2 Cytoplasmic inclusions (Melamed-Wolinska bodies). Degenerating urothelial cells frequently have round, eosinophilic (or sometimes green) cytoplasmic inclusions of varying sizes. A normal columnar-shaped urothelial cell is also present.

binucleation and multinucleation are common (see Fig. 3.3). Although large, umbrella cells have a low nuclear-to-cytoplasmic ratio, which helps to distinguish them from the cells of UC. Some squamous cells are common in voided urine samples; they exfoliate from foci of squamous metaplasia in the trigone of the bladder (a normal finding, especially in women). Squamous cells can also be picked up as urine passes through the urethral orifice and is contaminated by cells from the vagina. A voided urine sample with significant vaginal contamination (comprised almost exclusively of squamous cells and bacteria, the latter either normal flora or coccobacilli) may require catheterization to obtain a more pure specimen.³

In catheterized samples, including washings and brushings, clusters of urothelial cells, some quite large,

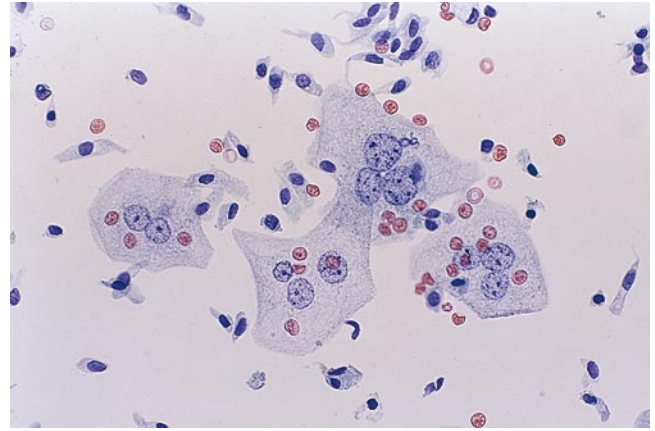
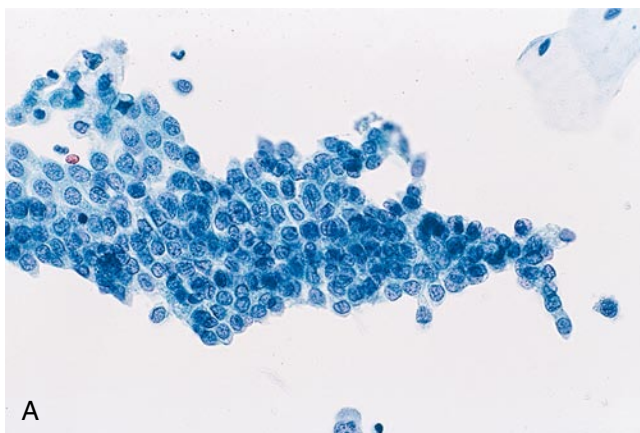


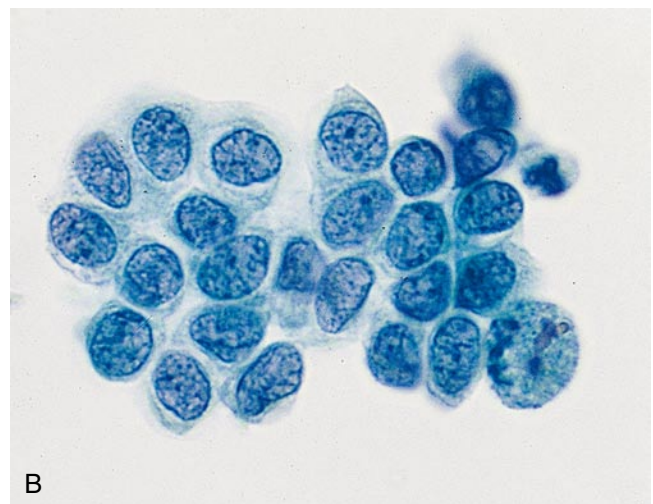
Figure 3.3 Umbrella cells. These are the largest urothelial cells and cover the surface of the urothelium. Normal columnar urothelial cells are also present.

are an entirely normal finding; the instrument mechanically abrades the mucosal surface, resulting in large numbers of cells in fragments. Thus, the entire spectrum of basal, intermediate, and superficial (umbrella) cells is seen. Normal catheterized specimens, particularly washings and brushings, may appear worrisome for malignancy, particularly to the novice cytologist, because of the presence of intact mucosal fragments and the marked polymorphism of the cell population. Umbrella cells alone may be worrisome because of their size; they are among the largest of human epithelial cells. Even the small basal urothelial cells (Fig. 3.4), because of their scant cytoplasm and dark nuclei, are also occasionally mistaken for carcinoma cells.

On rare occasions, seminal vesicle epithelial cells are seen in urine samples from male patients. They sometimes have hyperchromatic nuclei and can be mistaken for malignant cells. The clue to their benign nature is the presence of lipofuscin, a golden-brown cytoplasmic pigment (Fig. 3.5).



A



B

Figure 3.4 Basal urothelial cells (catheterized specimen). A, These cells are rare in voided urine but common in catheterized specimens and usually tightly clustered. B, Higher magnification reveals predominantly round, regular nuclear contours.

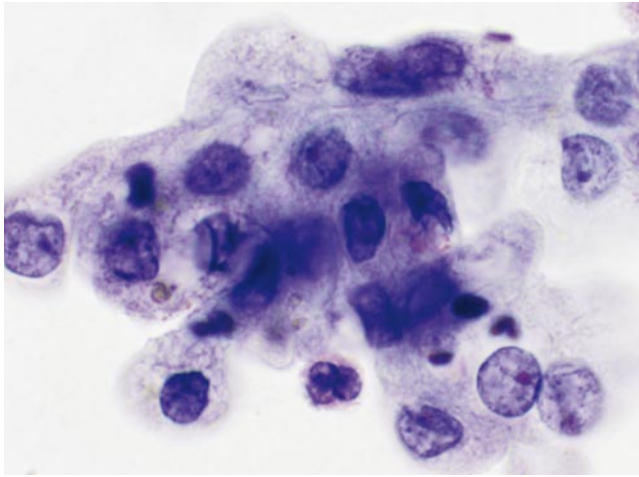


Figure 3.5 Seminal vesicle epithelial cells (voided urine). These cells are recognizable as seminal vesicle cells because of their golden-brown pigment. Sometimes they are less well-preserved than seen here.

The intestinal epithelial cells in ileal loop specimens are dispersed as isolated cells and show marked degenerative changes (Fig. 3.6), with eosinophilic intracytoplasmic inclusions such as those seen in degenerated urothelial cells. They are commonly mistaken for macrophages by the novice.

Crystals are commonly present. Occasional red blood cells are common, but the presence of many red blood cells is abnormal. Bacteria and *Candida* are commonly seen, usually without concomitant inflammation or clinical significance.

BENIGN LESIONS

Infections

Infections:

- bacteria, including malakoplakia
- fungi (especially *Candida*)
- herpes simplex virus
- cytomegalovirus (CMV)
- *Trichomonas vaginalis*
- polyomavirus
- human papillomavirus (HPV)

Infections of the bladder are caused by bacteria, viruses, parasites, or fungi. In the United States, bacterial infections are the most common. Cytologic preparations in cases of bacterial cystitis show a dense concentration of white blood cells (predominantly neutrophils), with plasma cells, lymphocytes, histiocytes, and numerous red blood cells. Bacteria are present, sometimes in overwhelming numbers. The inflammation can obscure the urothelial cells. The presence of bacteria in the absence of abundant neutrophils is nonspecific, usually the result of vaginal or urethral contamination. Malakoplakia is an uncommon histiocytic inflammatory lesion of the bladder or upper respiratory tract that results from bacterial infection. The cytologic hallmark is the presence of histiocytes whose abundant granular cytoplasm is filled with bacteria and bacterial fragments. Often one finds basophilic, round, lamellated bodies, known as

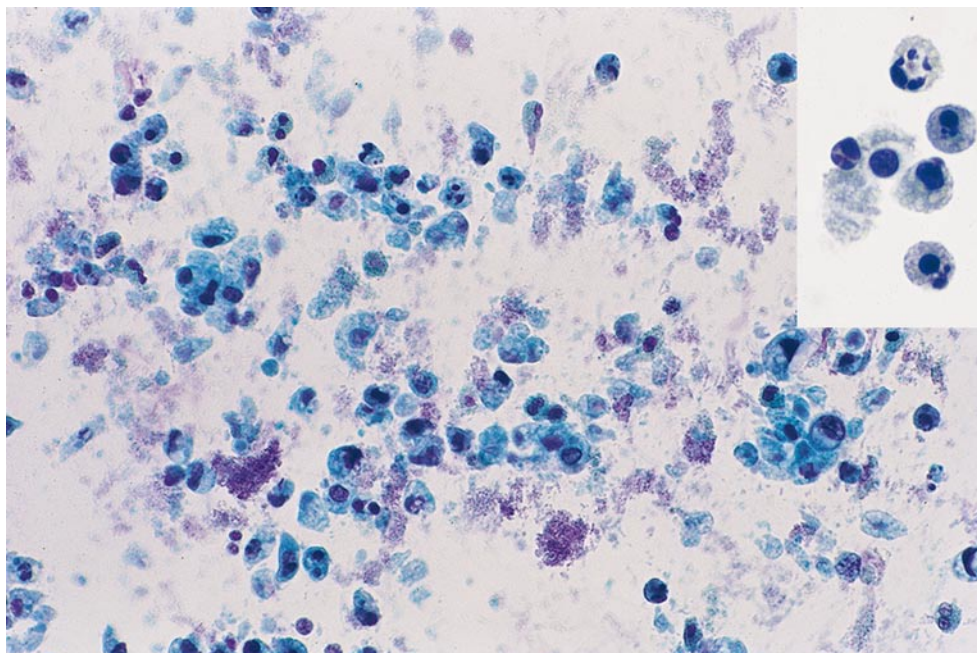


Figure 3.6 Ileal loop specimen. Most cells in ileal loop specimens are degenerated intestinal cells that resemble macrophages (inset).

Michaelis-Gutmann bodies, which measure approximately 8 μm and may be intracellular within histiocytes or extracellular.

The most common fungus that infects the bladder is *Candida*. The organism is present as a yeast form and as pseudohyphae and is accompanied by a cellular, mixed inflammatory background. Any urothelial cells that are present usually show reactive changes. When *Candida* is present in the urine of female patients, the possibility of vaginal contamination should be considered. Vaginal contamination, rather than true infection, is likely when the background contains numerous squamous cells and bacteria and few neutrophils.

Viral infections of the bladder include herpes simplex virus, cytomegalovirus, polyomavirus, and human papillomavirus. Herpetic infection of the bladder is uncommon; usually it is seen in the patient who is immunocompromised. The cytopathic changes include multinucleation, a ground-glass chromatin texture, and peripheral condensation of chromatin. In some cases the cells have a large eosinophilic nuclear inclusion, which may be sharply angulated. Nuclear molding is often observed. Infected cells can be greatly enlarged, bizarrely shaped, and have dense, opaque cytoplasm.

Cytomegalovirus affects urinary epithelium, most commonly renal tubular cells, in patients who are immunocompromised. Affected cells are markedly enlarged and have both nuclear and cytoplasmic inclusions. The nuclear inclusion is solitary, darkly basophilic, and is surrounded by a zone of chromatin clearing within the nucleus. More variable in appearance are the multiple cytoplasmic inclusions, which are smaller, basophilic, and either finely or coarsely granular.

The parasitic protozoan *Trichomonas vaginalis* is responsible for one of the most common sexually transmitted diseases. It is usually associated with vaginitis, but it can cause urethritis and even prostatitis. In urine from a woman, the organisms are most likely contaminants from a vaginal infection if they are accompanied by abundant squamous cells and vaginal flora.⁵⁶ Rarely, they cause urethritis in men and are identified in urine cytology from such patients.^{57,58} The organism varies in size, with an average length and width of 10 and 7 μm , respectively. The nucleus is small and oval, and the cytoplasm contains fine red granules.

The human polyomaviruses (JC and BK viruses), members of the papovavirus family, commonly infect urothelial cells in individuals who are both healthy and immunocompromised, and characteristic viral cytopathic changes are seen in 4% of urine samples.⁵⁹ The infection usually has no clinical significance, but infected cells appear atypical and can be confused with malignant cells.⁴⁶ Infected urothelial cells have large, eccentrically placed nuclei with basophilic nuclear inclusions that completely replace the nucleus and appear glassy, opaque, or cloudy (Fig. 3.7). Nuclear membranes are markedly thickened. In children and

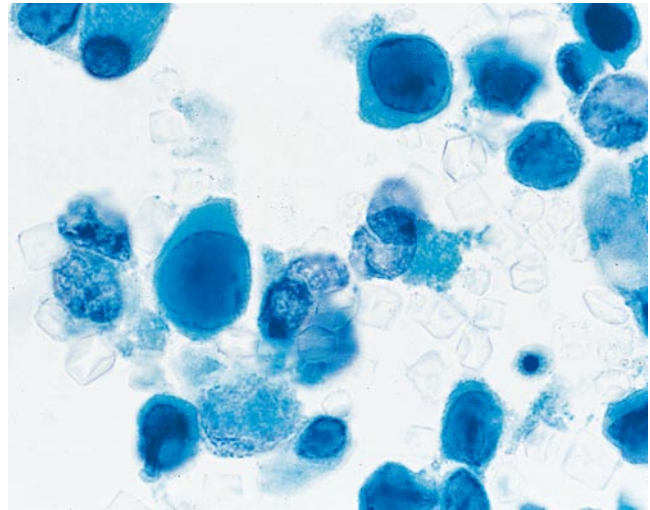


Figure 3.7 Polyomavirus infection. Some enlarged, round nuclei are virtually replaced by a glassy, homogeneous inclusion.

adults who are immunosuppressed, the altered cells are numerous, whereas in adults who are immunocompetent they are usually few in number.⁶⁰ In contrast to cytomegalovirus-infected cells, in which a halo surrounds the inclusion, the inclusion of polyomavirus fills the entire nucleus. Because of their increased nuclear size and hyperchromasia, polyomavirus-infected cells can be confused with malignant cells, hence their name “decoy cells.” Unlike most malignant cells, however, decoy cells have perfectly smooth and round nuclei. In contrast to tumor cells, which often cluster to form groups, polyomavirus-infected urothelial cells are found only as isolated cells.⁴⁶ Because degenerated UC cells can resemble decoy cells, however, one should not diagnose specimens as negative unless the morphology of the decoy cells is indeed classic.⁵⁹ The differential diagnosis of decoy cells also includes degenerated benign urothelial cells. Polyomavirus-infected nuclei appear smudged and densely basophilic, and the chromatin is more uniform in texture than that of degenerated urothelial cells.

Human papillomavirus can infect the urinary tract,⁶¹ but when cytopathic changes characteristic of this virus are seen in a voided urine specimen from a woman, the cells most likely have originated from the vulva or vagina. Koilocytes in a catheterized specimen, however, indicate a condyloma in the urinary tract.

Noninfectious Findings and Conditions

Noninfectious findings and conditions:

- crystals
- casts
- nonspecific reactive urothelial cell changes

- chemotherapy effect
- radiation therapy effect
- urothelial atypia associated with urinary calculi

Crystals

Crystals are a common finding in urine specimens. Most have no clinical significance, and their existence depends on the concentration of their constituents and on the pH and temperature of the specimen. Crystals are reported and classified as a part of routine urinalysis, which is carried out on wet preparations rather than on cytologic ones. Still, many crystals retain their characteristic shapes on alcohol-fixed, Papanicolaou-stained preparations. Triple phosphate crystals are shaped like prisms and resemble coffin lids. Ammonium biurate crystals are spheres with protruding spicules (“thorn apples”). Uric acid crystals are the most common. They vary markedly in size and shape, and may look like many other crystals. Calcium oxalate crystals can be oval, dumbbell shaped, or small and octahedral.

Pathologic crystals are much less common, and include those composed of bilirubin (brown granules and needles), cholesterol, cystine (hexagonal plates), leucine (spheres with radiating striations), and tyrosine (slender needles).

Casts

Casts found in urine samples may be of no clinical significance, or they may be a manifestation of serious renal disease. Hyaline casts and granular casts are physiologic and can be present in normal urine in large numbers, especially after physical stress. Hyaline casts have a homogeneous, glassy texture; granular casts are composed of finely or coarsely granular debris.

Red blood cell casts are characteristic of glomerular disease. White blood cell casts are seen in tubulointerstitial diseases and in association with transplant rejection. Epithelial casts, composed of degenerated renal tubular cells, can be seen in any disease, including acute tubular necrosis. Waxy casts are homogeneous and dense, often with sharp edges and fractures. Fatty casts contain lipid vacuoles and are seen in patients with the nephrotic syndrome.

Nonspecific Reactive Urothelial Cell Changes

Inflammation and injury to the urothelium result in reactive urothelial cell changes.

Cytomorphology of nonspecific reactive changes:

- enlarged nuclei
- prominent nucleoli
- coarsely vacuolated cytoplasm

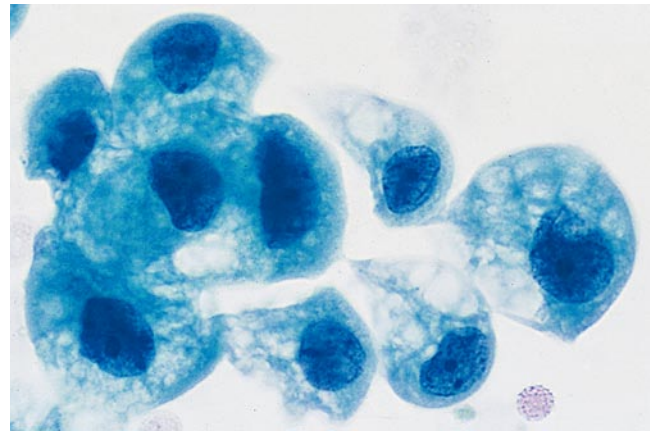


Figure 3.8 Reactive urothelial cells (catheterized urine). Coarsely vacuolated cytoplasm is characteristic of benign, reactive changes and uncommon in malignancy.

Cytologic features that support the diagnosis of reactive urothelial cells include coarsely vacuolated (as opposed to granular or finely vacuolated) cytoplasm, enlarged nuclei, and prominent nucleoli (Fig. 3.8). Although adenocarcinomas can have vacuolated cytoplasm, their nuclei are so atypical that they are rarely confused with reactive urothelial cells.

Effects of Radiation and Chemotherapy

Radiation typically produces cellular and nuclear enlargement, although the nuclear to cytoplasmic ratio is not increased overall. Affected cells are usually shed singly. The abnormalities produced vary greatly from one cell to the next. Cytoplasmic and nuclear vacuolization are common. Both cytoplasm and nucleus often show degenerative changes. The nuclear chromatin can be smudged and featureless, but the nuclear membrane remains smooth, without the irregularity typical of cancer cells. Radiation effect can persist for weeks, months, or years after completion of treatment.

Thiotepa and mitomycin C, topical agents injected intravesically for the chemotherapeutic management of bladder cancer, produce striking cellular abnormalities similar to those seen in epithelia after radiation. Affected cells have enlarged cytoplasm and nuclei, but the normal nuclear to cytoplasmic ratio is preserved.⁶² Nuclei are smooth in contour, round to oval in shape, and show chromatin smudging, but most affected nuclei are not hyperchromatic. Cells are frequently multinucleated, with vacuolization of nucleus or cytoplasm, which can have frayed borders. These changes have been observed in patients as early as 1 month after treatment with thiotepa.⁶³ Similar cytologic abnormalities are seen in patients receiving systemic chemotherapeutic agents such as cyclophosphamide and busulfan.

The changes induced by radiation and chemotherapy can mimic a UC.³⁹ Analysis of the nuclear-to-cytoplasmic ratio and nuclear chromasia is important in this regard;

although the nucleus of a normal urothelial cell is enlarged after radiation or chemotherapy, a normal nuclear-to-cytoplasmic ratio is preserved, and the nucleus is normochromatic. In recurrent cancer, the nucleus is dark and the nuclear-to-cytoplasmic ratio is increased.

Urothelial Atypia Associated with Urinary Calculi

Urinary tract calculi are the most frequent cause of ureteral obstruction. Patients present with hematuria, sometimes accompanied by severe pain. Large stones can be visualized on imaging studies. Some cytologic specimens have a background of blood. Inflammatory cells can be present, including neutrophils and lymphocytes. Tight groups of urothelial cells are common. In most instances these groups have smooth contours and the cells appear benign; they have a centrally placed nucleus with a smooth nuclear border and finely granular chromatin. Such innocuous-looking cell clusters are impossible to distinguish from those of a papilloma, a papillary urothelial neoplasm of low malignant potential, or even some low-grade UCs. In a small proportion of patients with stones, the urothelial cells are atypical, some forming irregular and dyshesive clusters (Fig. 3.9). They can show pleomorphism, coarsely granular chromatin, hyperchromasia, irregular nuclear staining, and occasional mitotic figures, and can lead to a false-positive cytologic diagnosis.^{45,64,65}

UROTHELIAL NEOPLASMS

For unclear reasons, the incidence of urothelial carcinoma is rising. It accounts for 3% of all cancer-related deaths;⁶⁶⁻⁷⁰ there are 67,100 new cases of bladder cancer in the United States each year and 13,750 deaths/year.⁷⁰ UC occurs three times as often in males, usually

in patients over 50 years of age, and is associated with smoking and other risk factors, including a number of occupational exposures.^{2,66} Patients receiving cyclophosphamide, used for the treatment of nonurologic diseases like multiple sclerosis and Wegener granulomatosis, are at increased risk for developing bladder cancer.¹³ UCs typically have a large number of genetic alterations involving multiple different chromosomes.

Occupational and environmental exposures and other risk factors for urothelial neoplasms:

- aromatic amines
- phenacetin
- cyclophosphamide treatment
- alkylating agents
- smoking
- *Schistosoma hematobium*
- paralysis
- exstrophy
- diverticula

Several different histologic classification systems for urothelial neoplasms have been used in the past. The current standard is that of the World Health Organization (WHO) and the International Society of Urologic Pathologists (ISUP).⁶⁹ Many pathologists, however, continue to use the prior WHO classification system, which divided bladder cancers into three grades: transitional cell carcinomas grade 1, 2, and 3. The new WHO/ISUP system recognizes a new entity, the papillary urothelial neoplasm of low malignant potential (PUNLMP). Many of the lesions formerly called *transitional cell carcinoma, grade 1* are now considered PUNLMPs and are therefore not, strictly speaking, carcinomas. Most of the formerly termed *transitional cell carcinomas grade 2* are now considered either low-grade or high-grade UC.

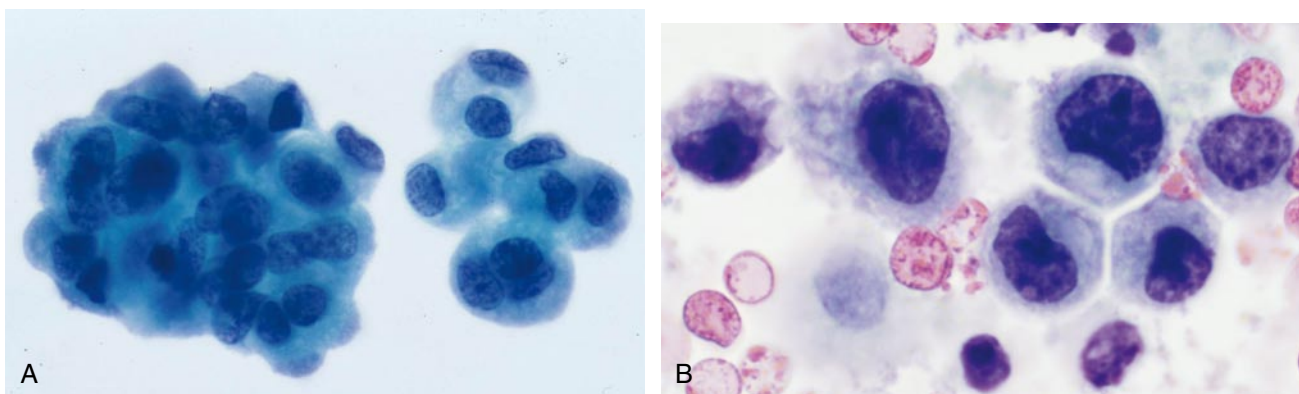


Figure 3.9 Benign stone atypia. A, The mild atypia of these urothelial cells was induced by multiple bladder stones, which were removed 4 days later. Subsequent urines have been benign. B, In some cases, the urothelial cells are markedly atypical, with hyperchromatic and angulated nuclei. A distinction from urothelial carcinoma in such cases can be impossible.

Current World Health Organization and International Society of Urologic Pathologists classification system for urothelial neoplasms:

- flat lesions
 - dysplasia
 - carcinoma in situ
- papillary lesions
 - papilloma
 - PUNLMP
 - low-grade UC
 - high-grade UC

Several points deserve emphasis regarding the current histologic classification system for bladder tumors. First, papillomas, defined histologically as papillary lesions with no cytologic atypia, are rare and occur almost exclusively in young patients. Similar lesions in older patients likely represent PUNLMPs, which are defined histologically as papillary lesions with minimal to absent cytologic atypia but an increased cellular proliferation exceeding the thickness of normal urothelium. Second, PUNLMPs are so designated to reflect the low risk (10% or less) of progression to invasive disease. PUNLMPs comprise approximately one third of papillary tumors. Third, urothelial dysplasia is a controversial, poorly defined entity, and the histologic and cytologic interpretation of dysplasia is irreproducible. For these reasons, an interpretation of “dysplasia” in a cytologic preparation of urine should be avoided. Because histologic dysplasia, such as it is, is almost always accompanied by CIS, its clinical significance is unclear. Finally, the threshold for the diagnosis of a high-grade tumor is set lower than in previous classifications.

The current WHO histologic classification system has important implications for cytologic diagnosis. The sensitivity of cytology for detecting malignancy has theoretically improved with the adoption of the current system because a lesion previously interpreted histologically as a grade 1 transitional cell carcinoma is now considered a PUNLMP. (Conversely, the precision and accuracy of cytology for the detection of papillomas and PUNLMPs, although not well studied, can be presumed to be poor by analogy to older data on grade 1 transitional cell carcinomas.) Most importantly, in the new classification system the term *low-grade carcinoma* is used for some lesions that in the older system were classified as transitional cell carcinoma, grade 2. This has led to some confusion because, for the sake of simplicity, grade 2 and 3 transitional cell carcinomas were sometimes lumped together and referred to as “high-grade” carcinomas. It is now more important than ever that cytologists, surgical pathologists, and urologists all speak a common language!

The stage of disease plays an important role in determining treatment. Superficial bladder cancers (i.e., non-invasive tumors or tumors that invade no deeper than

the lamina propria) most often are treated conservatively and are followed at regular intervals (e.g., every 3 months) with cystoscopy and cytology for recurrence and progression. Patients with a UC that has invaded into the muscularis propria (“muscle-invasive bladder cancer”) are candidates for cystectomy.

Papilloma

Although there remains considerable debate, most investigators believe that a papilloma is best defined as a rare papillary lesion without any cytologic atypia that occurs almost exclusively in young patients and never recurs. Because the lesion has no atypia, it cannot be recognized cytologically unless an intact papillary frond is identified.

Low-Grade Urothelial Lesions

Papillary Urothelial Neoplasm of Low Malignant Potential and Low-Grade Urothelial Carcinoma

In the latest WHO classification system, a PUNLMP is defined histologically as a papillary lesion with minimal to absent cytologic atypia but an increased cellular proliferation exceeding the thickness of normal urothelium. A low-grade UC is a neoplasm with mild nuclear atypia. Because the latest WHO classification system is new, few studies on its application to cytology have been published. In the old WHO classification system, lesions now diagnosed as PUNLMP and low-grade UC were often diagnosed as grade 1 or 2 UCs, respectively. The cytologic features of these lesions are similar, except that PUNLMPs have less atypia; one expects PUNLMPs to be more difficult to recognize cytologically than low-grade carcinoma. For the purpose of this discussion, however, these two lesions are discussed together as the low-grade lesions.

The criteria for diagnosing low-grade lesions on cytologic material have been extensively studied over the past two decades. There are two general approaches: cytologic (Fig. 3.10) and architectural (Fig. 3.11). The cytologic criteria^{41,70,71} are listed in the following box.

Cytologic criteria for diagnosing low-grade lesions:

- cytoplasmic homogeneity
- high nuclear-to-cytoplasmic ratio
- irregular nuclear membranes

Although there is little argument over the criteria, there is disagreement on how accurately low-grade lesions can be diagnosed. The sensitivity of cytology for detecting low-grade tumors, using these criteria, is about 30%, and the specificity about 80%.⁷¹ In practice, specimens from patients without tumors far outnumber

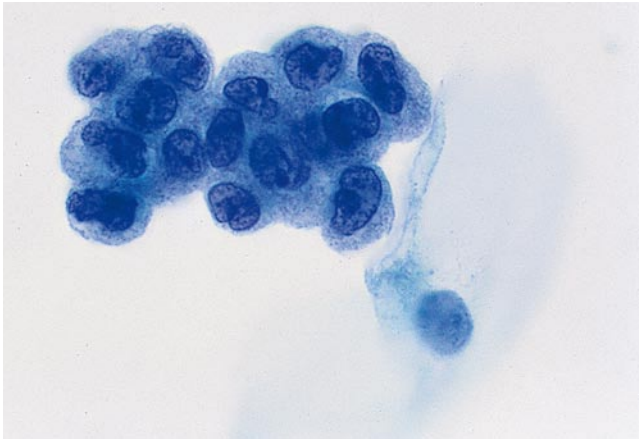


Figure 3.10 Cytologic criteria for the diagnosis of a low-grade urothelial lesion (catheterized specimen). Homogeneous cytoplasm, an increased nuclear-to-cytoplasmic ratio, and irregular nuclear outlines are associated with low-grade lesions, but are not specific.

those from patients with tumors. As a result, most patients with a cytologic diagnosis of a low-grade lesion prove not to have a tumor.

The architectural criteria⁷¹⁻⁷⁵ include the features listed in the following box.

Architectural criteria for diagnosing low-grade lesions:

- papillary fragments with fibrovascular cores (diagnostic, but rare)
- cell clusters without cores (not specific; also seen with urolithiasis, catheterization)
- irregular cell clusters (more commonly associated with UC than smooth cell clusters)

A papillary fragment with an intact fibrovascular core is diagnostic, but finding one is rare, occurring most often when urine is collected after a urologist has biopsied

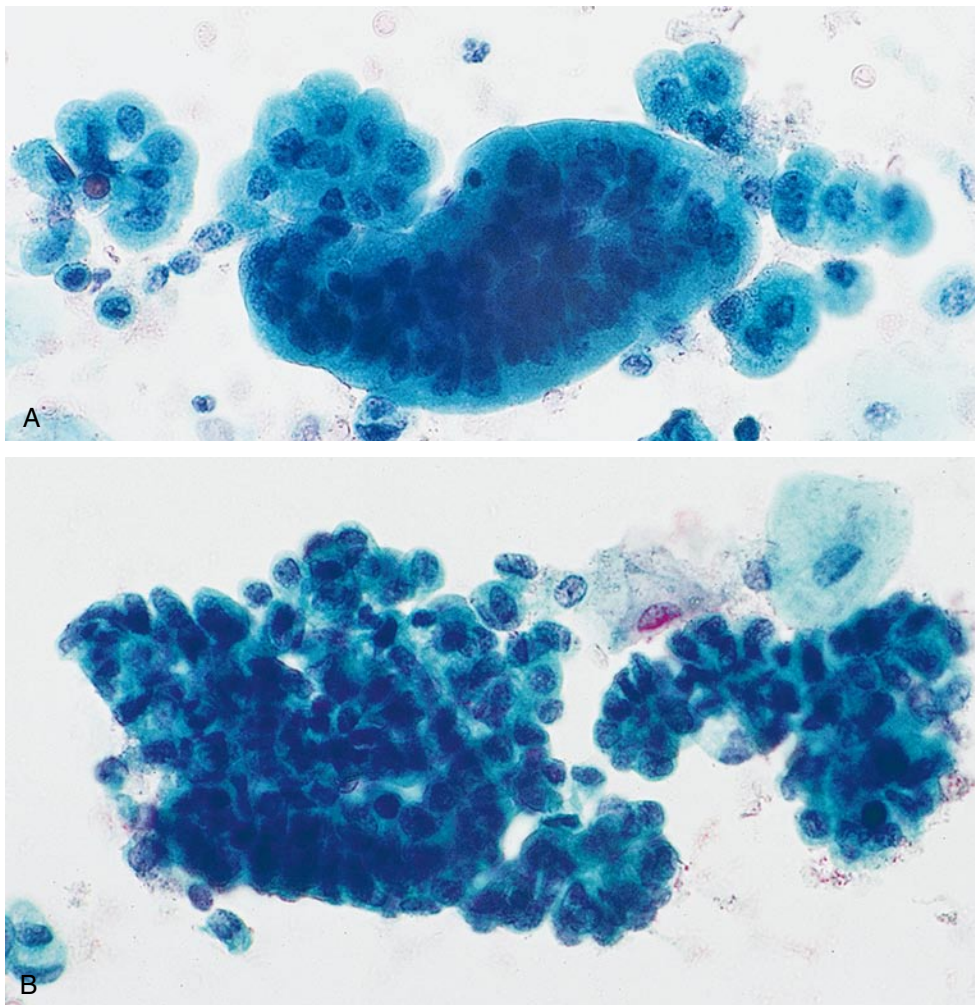


Figure 3.11 Architectural criterion for the diagnosis of a low-grade urothelial lesion (catheterized specimen). Unlike the smooth outline ("collared" groups) of benign clusters A, low-grade lesions tend to exfoliate as clusters with irregular edges B. This feature lacks good specificity, however.

an obvious lesion. Numerous irregular fragments are associated with a sensitivity of 10% in voided urine and 44% in catheterized urine. The specificity is 83% in voided urine and 69% in catheterized urine. False-positive results are frequent if one uses these criteria to diagnose a low-grade lesion, because benign changes such as stone atypia mimic low-grade lesions (see Fig. 3.9A).

The cytologic and architectural criteria are unreliable in practice; these lesions have little chance of progression; and the urologist usually can see these lesions cystoscopically. For these reasons, there is little justification for the cytologic diagnosis of low-grade lesions. There is one setting in which urologists would very much wish cytologists could do it: in the upper urinary tract (ureter and renal pelvis), where cystoscopic visibility is markedly reduced. But here the criteria, unfortunately, have proven even less useful.

Although these criteria are not good for identifying low-grade tumors, they are helpful in designating a reactive pattern.

Dysplasia

Although listed in the new WHO histologic classification system, dysplasia is a controversial lesion. There is no consensus on its morphologic features (opinions range from normal to high-grade nuclei). In addition, the diagnosis of dysplasia is of limited value in cytology because almost all patients have a coexistent high-grade lesion. Finally, the term lacks precision in cytology because some cytologists use it to refer to a specific

entity, whereas others use it in descriptive fashion for any atypia that is potentially neoplastic. Until greater agreement can be reached, it is advisable to avoid the term *dysplasia* in urinary cytology.

High-Grade Urothelial Carcinoma and Carcinoma in Situ

In the new WHO classification system, high-grade UC is defined histologically as a tumor with moderate-to-marked cytologic and architectural atypia; it can be an invasive tumor or a papillary, noninvasive tumor. Urothelial CIS is a flat, noninvasive lesion confined to the epithelium and comprised of cytologically malignant cells. CIS and high-grade UC are indistinguishable cytologically and are considered together in the following discussion.

CYTOMORPHOLOGY OF CARCINOMA IN SITU AND HIGH-GRADE UROTHELIAL CANCER:

- high nuclear-to-cytoplasmic ratio
- marked nuclear hyperchromasia
- coarsely granular chromatin
- irregular nuclear outline
- large nucleoli (some cases)

Urine and bladder washings contain predominantly isolated cells (Fig. 3.12), although occasional cell groups are seen (Fig. 3.13). The cells are large and highly atypical, often with a high nuclear-to-cytoplasmic ratio.

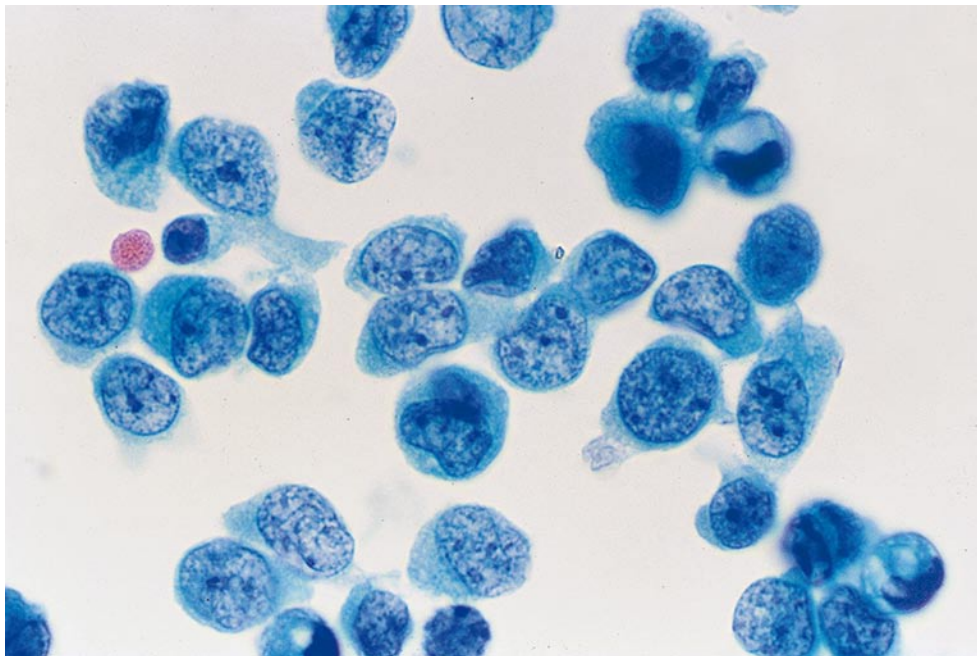


Figure 3.12 High-grade urothelial carcinoma (UC). Numerous isolated malignant cells have enlarged, dark nuclei and an increased nuclear-to-cytoplasmic ratio.

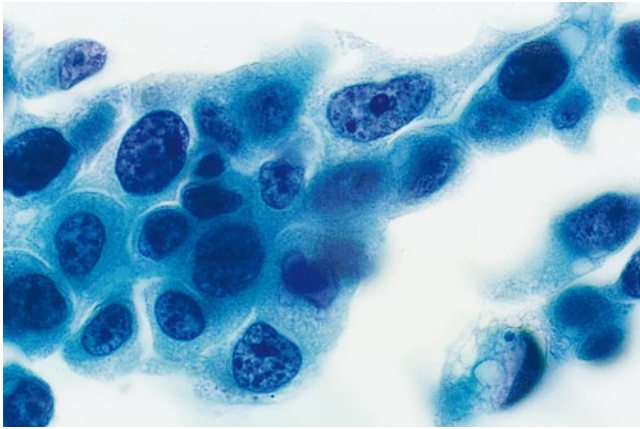


Figure 3.13 High-grade urothelial carcinoma (UC). A cluster of malignant urothelial cells, some with prominent nucleoli.

Nuclei are hyperchromatic, with coarsely granular chromatin and irregular nuclear membranes; enlarged and angular nucleoli are seen in some cases. Some malignant cells contain Melamed-Wolinska bodies.⁵⁴ The background can contain necrotic debris, blood, and inflammatory cells and does not aid in distinguishing in situ from invasive tumors. High-grade UCs can show squamous or glandular differentiation (Fig. 3.14).

In most cases the diagnosis is straightforward. The sensitivity of urine cytology for high-grade UC is 79%, and the specificity is greater than 95%.³⁹

DIFFERENTIAL DIAGNOSIS OF CARCINOMA IN SITU AND HIGH-GRADE UROTHELIAL CARCINOMA:

- polyomavirus
- stone atypia
- normal upper tract washings or brushings
- treatment effect
- nonspecific reactive changes

Because of their increased nuclear size and hyperchromasia, polyoma virus-infected cells (decoy cells) can be confused with malignant cells, particularly high-grade UC (see Fig. 3.7). Malignant nuclei, however, are rarely as perfectly round as decoy cell nuclei. In contrast to tumor cells, some of which form groups, polyomavirus-infected urothelial cells are found only as isolated cells.⁴⁶ Kidney and bladder stones cause irritation of the urothelium, which in some cases results in a marked urothelial cell atypia that mimics UC⁴⁵ (see Fig. 3.9b). If the patient is known to have a stone, a conservative approach to diagnosis is warranted.

Normal upper tract (ureteral and renal pelvic) washings are prone to false-positive diagnosis.²³ The cells are numerous, with large nuclei and a high nuclear-to-cytoplasmic ratio. Bilateral specimens are helpful because they allow comparison between a lesional and presumably normal specimen (Fig. 3.15). Preparing a cell block from the residual sediment is also particularly useful in this setting.¹⁹

Radiation and chemotherapy produce cellular and nuclear enlargements that suggest UC, but the nuclear-to-cytoplasmic ratio is not increased, and there is generally no significant hyperchromasia. There can be multinucleation, and cytoplasmic and nuclear vacuolization are common. The nuclei of treatment effect are sometimes smudged and featureless, but some UCs can have similar features.

Coarse cytoplasmic vacuolization is very uncommon in UC. When present, it is a clue that the atypia is as a result of benign reactive changes (see Fig. 3.8) rather than malignancy.

Urinary cytology is sometimes positive in the absence of a visible lesion.⁴⁷ There are two possible explanations. The lesion may be in the upper tract, for which radiologic studies and selective sampling are indicated. Alternatively, a bladder lesion might be cystoscopically subtle or undetectable, in which case

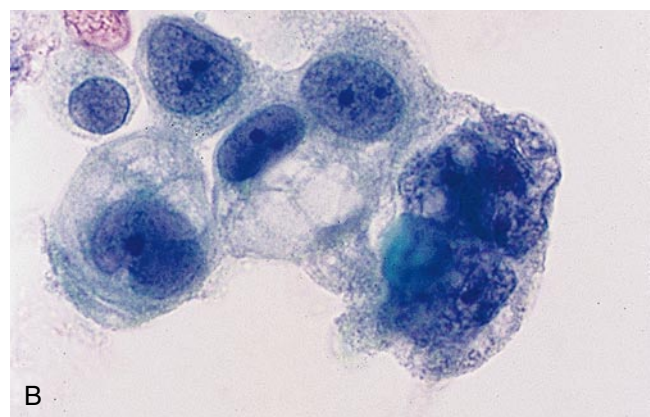
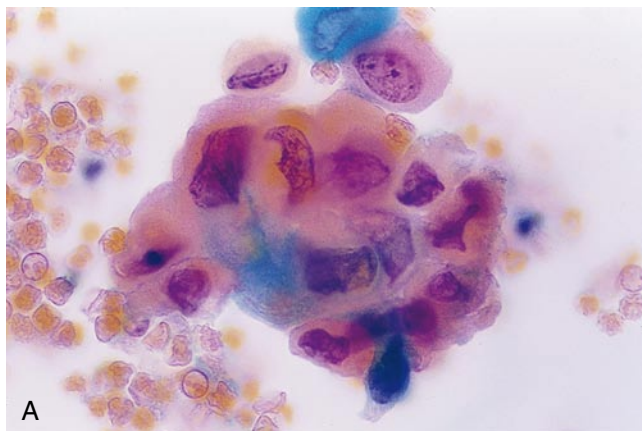


Figure 3.14 Urothelial carcinoma (UC) variants. A, Some of the malignant cells show squamous differentiation, manifested by cytoplasmic orangeophilia. B, Some UCs have foci of adenocarcinoma.

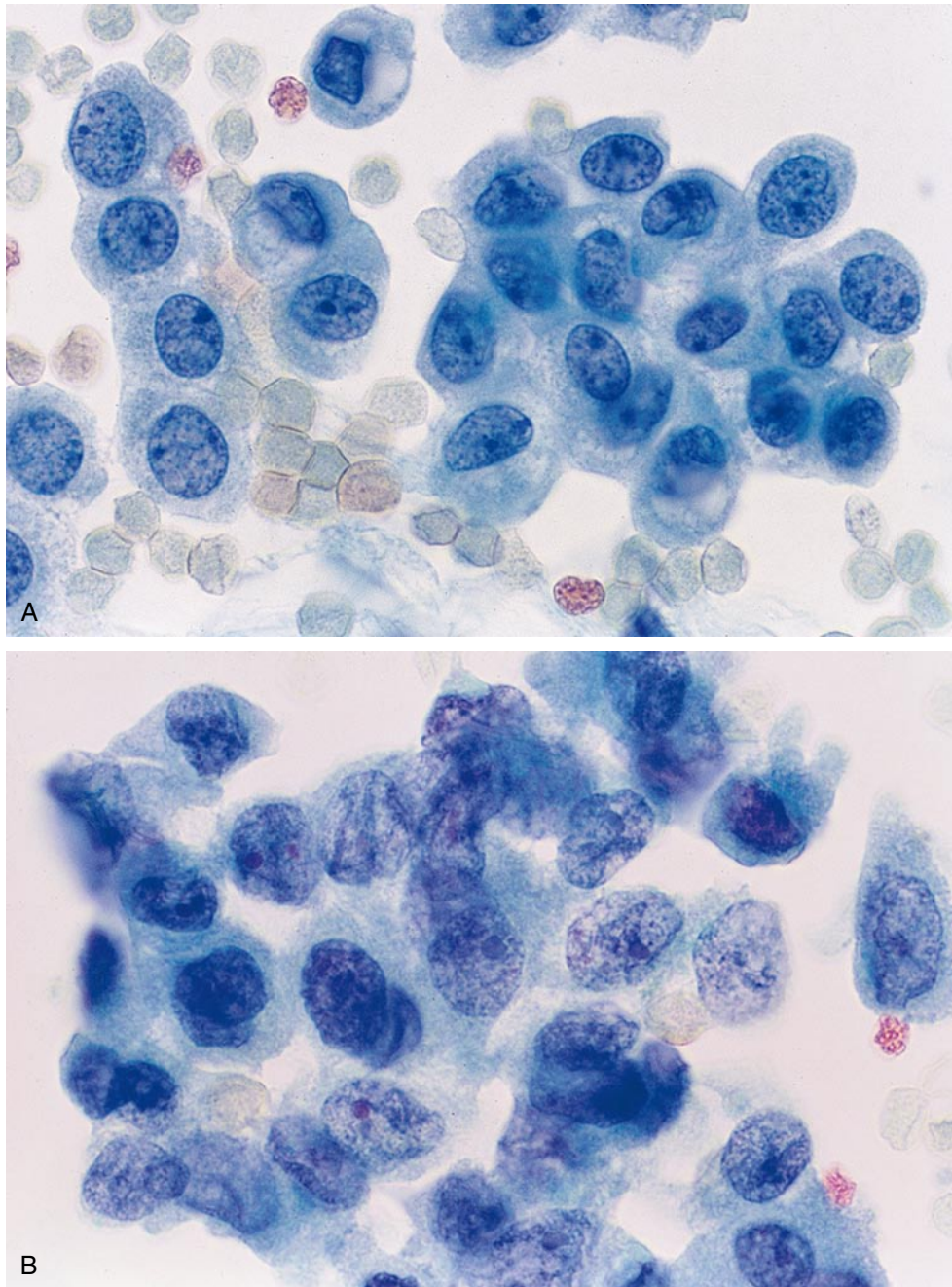


Figure 3.15 Bilateral ureteral washings. A, A sample from the left ureter shown benign urothelial cells. B, A sample from the right ureter contains malignant cells from a urothelial carcinoma (UC).

blind biopsies can be of value. Even if blind biopsies are negative, a lesion is usually detected within the next few years.

Following treatment for UC, patients require continued surveillance for lesions that might develop elsewhere in the bladder, upper urinary tract, or urethra, given the multifocal nature of urothelial neoplasia. A patient whose urethra has not been removed at the time of radical cystectomy is screened by periodic urethral irrigation or swabbing.

OTHER MALIGNANT LESIONS

Other Primary Cancers of the Urinary Tract

Squamous Cell Carcinoma

Pure squamous cell carcinoma (SQC) is rare and strongly associated with *Schistosoma hematobium*. It is common in the Nile River valley but rare in the United States

(<3% of all bladder cancers). Importantly, focal squamous differentiation is common in urothelial carcinomas (see Fig. 3.14A). A definite diagnosis of squamous cell carcinoma should be deferred to biopsy or resection.

CYTOMORPHOLOGY OF SQUAMOUS CELL CARCINOMA:

- cytoplasmic keratinization
- pearls
- bridges
- angulated hyperchromatic nuclei

The differential diagnosis includes condyloma acuminatum of the bladder,⁶¹ metastatic squamous cell carcinoma, and a squamous cell carcinoma of the gynecologic tract.

Adenocarcinoma

Adenocarcinomas of the bladder are rare and strongly associated with bladder exstrophy and urachal remnants. They represent less than 2% of all bladder carcinomas. They resemble gastrointestinal adenocarcinomas, either well-differentiated tumors with isolated columnar cells, hyperchromatic nuclei, and amphophilic cytoplasm or poorly differentiated tumors with signet ring cells or high-grade nuclei with prominent nucleoli. Abundant mucin can be present. Glandular differentiation is common in otherwise typical UCs (see Fig. 3.14B), and a definitive diagnosis of pure adenocarcinoma is best left to biopsy or resection. The differential diagnosis includes metastatic adenocarcinoma, particularly from the rectum.

Clear Cell Carcinoma

Clear cell carcinoma of the bladder is extremely rare⁷⁶ and resembles clear cell carcinoma of the female genital tract. When it occurs in the urologic tract it can be related to müllerian remnants, and it arises more often in or near the urethra than in the bladder.

CYTOMORPHOLOGY OF CLEAR CELL CARCINOMA:

- small rounded clusters of obviously malignant cells
- abundant clear cytoplasm
- large irregular nuclei
- vesicular chromatin
- large nucleoli

Small Cell Carcinoma

Small cell carcinoma is a rare aggressive tumor, although the prognosis for patients is better than for those with small cell carcinoma of other sites.⁷⁷ The cytomor-

phology^{78,79} is identical to that of small cell carcinoma of other sites. The differential diagnosis includes metastatic lesions, especially from the lung.

Metastatic Cancers

Renal Cell Carcinoma

Some investigators have found malignant cells in the urine of approximately 50% of patients with renal cell carcinoma (RCC), including those with small tumors, but these studies predate the widespread use of computed tomography (CT) and magnetic resonance imaging (MRI).⁸⁰ This high detection rate is at odds with other reports,⁸¹ and the personal experience of the author, who has reviewed the records from one large medical center over 10 years and found no such cases. Even those who more commonly detect renal cell carcinoma cells in urine acknowledge that urine cytology has no value in screening for renal cell carcinoma.^{16,80}

Prostatic Carcinoma

Prostatic carcinoma cells in urine specimens almost always occur in patients with poorly differentiated (Gleason score ≥ 8), unresectable tumors. There are two characteristic appearances (Fig. 3.16). In some cases, the cells have prominent nucleoli and relatively abundant cytoplasm and are easy to identify as prostatic in origin.⁸² In other cases, the cells have dark nuclei and resemble urothelial carcinoma⁸¹; without clinical information, the correct diagnosis is difficult to achieve. Fortunately, the diagnosis of prostate cancer is already known in most cases. Nevertheless, in some cases the diagnosis is first suggested by urine cytology.⁸²

Colonic Carcinoma

Colonic carcinoma cells in urine specimens are often indistinguishable from those of a UC. The cells are pleomorphic, with degenerated, hyperchromatic, irregular nuclei, and some cells can be vacuolated.⁸¹ A clinical history of colon cancer might alert the cytologist to the possibility of metastatic colon cancer; cell block sections can be used to identify the characteristic immunophenotype of the cells (Fig. 3.17).

DIAGNOSING DIFFICULT OR BORDERLINE SPECIMENS: COMMON PATTERNS

Not surprisingly, the diagnosis of “atypia” is used with great freedom in urine cytology; many specimens show degenerative changes, and worrisome reactive changes are common. Although an atypical diagnosis appears

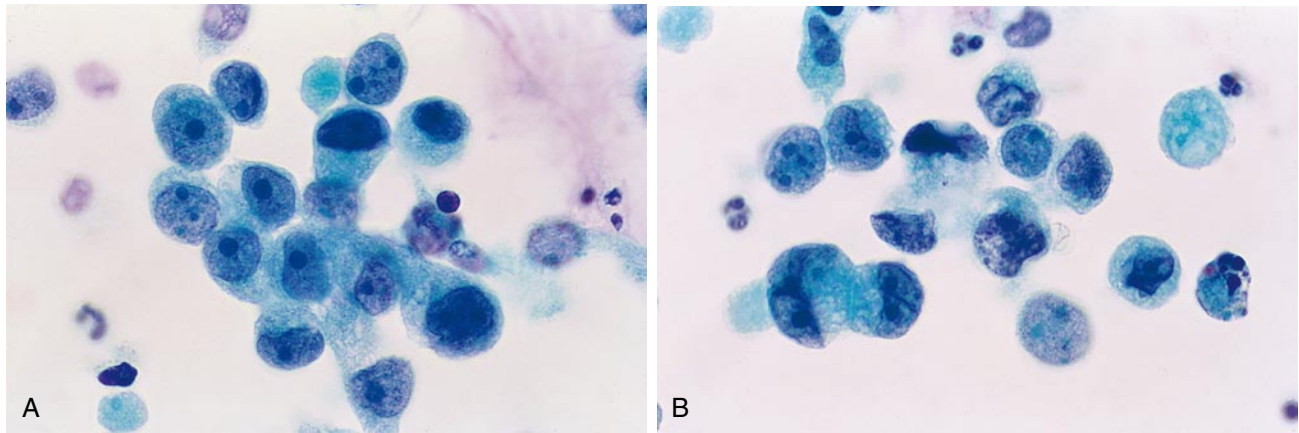


Figure 3.16 Prostatic carcinoma. A, Some tumors have abundant cytoplasm and large nucleoli. B, Other high-grade prostatic cancers are hard to distinguish from urothelial carcinoma (UC).

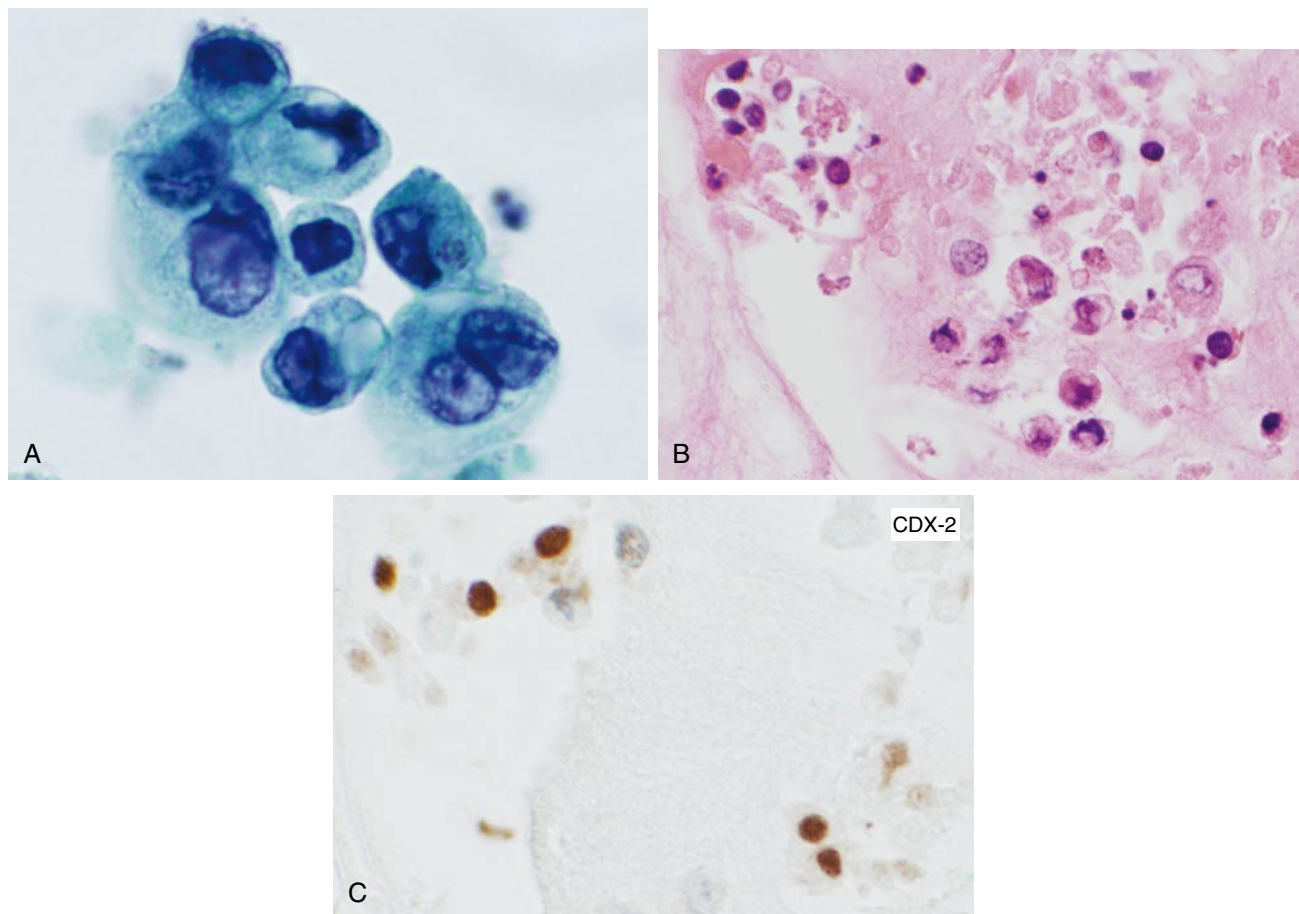


Figure 3.17 Metastatic colon cancer to the bladder neck. A, The majority of the malignant cells are round, with dark, angulated nuclei. Cytoplasmic fragments from necrotic cells are present. Distinction from urothelial carcinoma (UC) is not possible by conventional morphology. B, A cell block preparation contains occasional degenerated malignant cells. C, The malignant cells show nuclear reactivity for CDX-2, commonly seen in colon cancers and usually absent in UCs. The malignant cells were also positive for cytokeratin 20 and negative for cytokeratin 7, the typical keratin profile of colon cancer.

wise from a clinical and risk management point of view, this may not be the case; atypical specimens are usually regarded by urologists as negative. For this reason, it is advisable to use atypical as sparingly as possible

by classifying such cases instead as either negative or suspicious.

Most atypical urine specimens can be classified into one of five patterns.

Common patterns of atypical urine specimens:

- cell clusters in voided urine: diagnose as negative
- cytologic or architectural criteria for a low-grade lesion: diagnose as negative
- rare small highly atypical cells: diagnose as suspicious
- degenerated atypical cells with intact nuclear outlines: diagnose as suspicious
- rare mildly atypical cells: try to diagnose as negative

Although it is traditional teaching that clusters of urothelial cells in a voided urine sample are associated with an increased risk of a low-grade urothelial neoplasm, data to support this contention are sparse.^{83,84} Anecdotal cases have been described, but, in most studies, clusters of cells in voided urine specimens are no more common in patients with tumors than in those without tumors. For this reason, it is advisable to diagnose such specimens as negative. If clusters of urothelial cells are particularly numerous, an explanatory comment such as the following can be helpful: “Clusters of minimally atypical urothelial cells are present. The underlying risk for UC is not well established. The finding is not specific and can be seen in a variety of conditions, including urolithiasis.”

The second pattern consists of the constellation of features suggestive of a PUNLMP and low-grade UC. As discussed previously, the criteria are not accurate. Most urologists do not expect cytologists to diagnose these lesions, and their risk of progression to a high-grade UC is so low that the value of this cytologic diagnosis is debatable. It is more important for a cytologist to focus on identifying subtle patterns of high-grade tumors. For a negative urine sample interpretation, some laboratories,

albeit a minority, use the general heading “negative for a high-grade malignancy” rather than “negative for malignant cells” to reinforce the main purpose of urine cytology.

The next two patterns are associated with the greatest risk of a high-grade tumor. Although most high-grade tumors are easy to diagnose, some are more difficult. In one subtle pattern, the malignant cells are rare, small, sometimes hypochromatic, and occasionally obscured by blood. They have been termed *coy cells*.⁸⁵ A helpful clue to their malignant nature is significant nuclear membrane irregularity (Fig. 3.18). They are analogous to atypical squamous metaplastic cells of the cervix, which, although difficult to identify, are suspicious for a high-grade squamous intraepithelial lesion. Specimens with coy cells should be diagnosed as suspicious rather than atypical.

Another worrisome pattern is that of degenerated cells. If the entire specimen is poorly preserved, it is uninterpretable and should be diagnosed as inadequate. On the other hand, degenerative changes are common in high-grade tumors, resulting in dark, smudgy chromatin^{83,84} (Fig. 3.19). If some cells have an intact, irregular nuclear membrane, and benign cells in the background are well preserved, the possibility of a high-grade lesion cannot be excluded, and the specimen should be diagnosed as suspicious rather than atypical.

Finally, there is the urine specimen that contains only a few cells with enlarged, slightly irregular nuclei. This is the most common (and frustrating!) of the patterns. If these cells are few and the changes mild, the specimen should be diagnosed as negative. Although one would like to avoid an atypical diagnosis as much as possible, this is not always possible, and even experienced cytologists find the atypical category at times unavoidable.

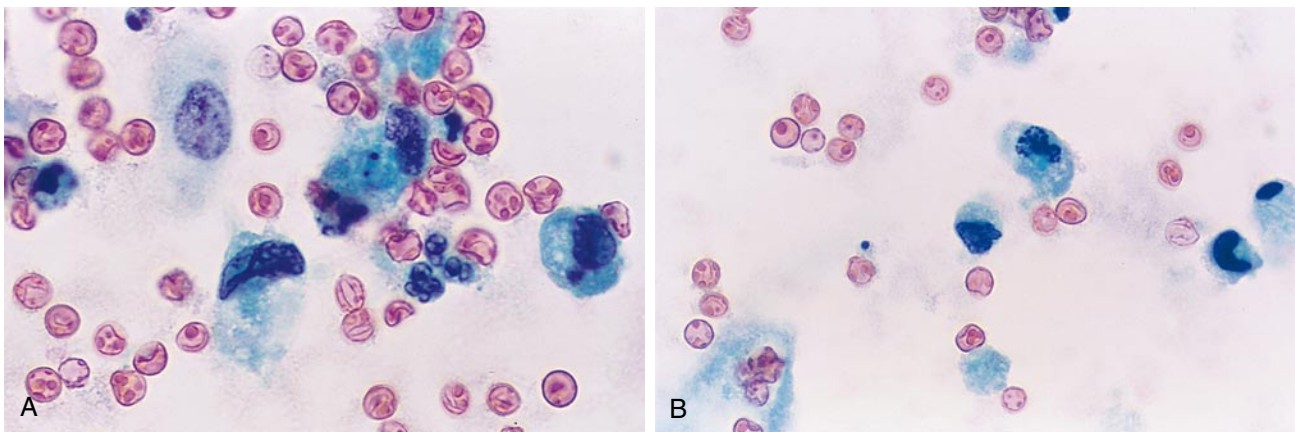


Figure 3.18 High-grade urothelial carcinoma (UC), difficult to detect. A and B, In some cases, the malignant cells are hyperchromatic but rare and hidden by blood (“coy cells”). Nuclear outline irregularity is marked.

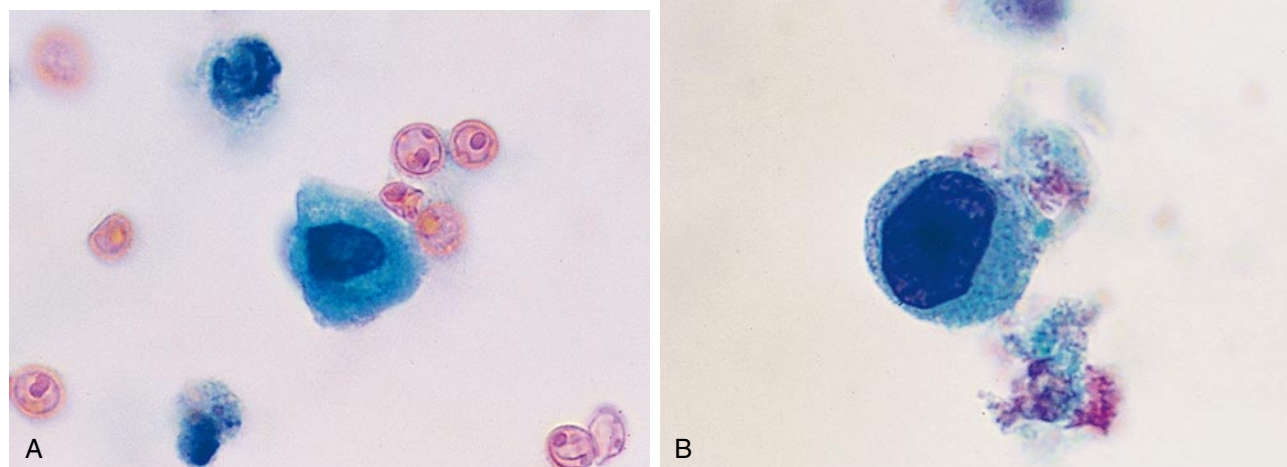


Figure 3.19 Degenerating cells of a high-grade urothelial carcinoma (UC). A and B, Although the chromatin is smudgy, the nuclear membrane is intact. Such cells should not be ignored.

ANCILLARY TECHNIQUES

Ancillary techniques:

- DNA aneuploidy (flow cytometry, image analysis)
- Bard bladder tumor antigen (BTA)TM test
- nuclear matrix protein NMP22 test
- telomerase assays
- microsatellite instability assays
- hyaluronidase and hyaluronic acid
- growth factors
 - acidic fibroblast growth factor (FGF)
 - basic FGF
 - autocrine motility factor
 - epidermal growth factor
 - transforming growth factor- β
- cell adhesion molecules
- fibrinogen degradation products
- tumor-associated and blood group antigens
- FISH

Great efforts have been made to develop a test that either improves on cytology in detecting UC or better predicts progression of a UC.^{2,66,86,87} A partial list of commercially available or investigational tests is given in the preceding box. The hope is that a more accurate test will eliminate the need for cystoscopy, which is costly and uncomfortable, in the follow-up of patients with conservatively treated, superficial UCs.

Many of these tests have greater sensitivity than cytology in detecting UC, but their less than ideal specificity remains a problem.⁸⁸ Like cytologic examination, these tests have difficulty distinguishing reactive conditions, such as stone-induced urothelial atypia, from UC.

A test based on our growing understanding of the genetic changes associated with UC, however, shows promise.⁸⁹⁻⁹³ Deletion of the p16 gene at chromosome 9p21 is one of the most common early alterations in UC, and tumor progression is associated with aberrations of chromosomes 1, 3, 7, 9, 11, 17. FISH technology can be applied to cytologic preparations to detect such cytogenetic abnormalities. Because no single abnormality is present in all UCs, the success of the technique depends on using several probes. One multitarget FISH assay, the UroVysion testTM (Abbot/Vysis, Downers Grove, Ill.), combines centromeric probes to chromosomes 3, 7, and 17 with a locus-specific probe to band 9p21 (Fig. 3.20). UroVysionTM has been approved for the surveillance of patients treated for UC and as a screening tool in patients with hematuria.^{40,94} In the pre-clinical trials for Food and Drug Administration (FDA) approval, the sensitivity of the test for low-grade lesions (transitional cell carcinoma grade 1) ranged from 48% to 61% (specificity 88% to 95%) and for high-grade lesions (transitional cell carcinoma grade 3) from 88% to 93% (specificity 80% to 95%). The method is reportedly more accurate than concurrent cytology, although the performance of cytology in these trials⁴⁰ was significantly worse than has been reported in almost all previous large series (see Table 3.2).

Several aspects of the UroVysionTM test deserve comment. First, the performance of the test has varied considerably among laboratories.⁹⁵⁻¹⁰² Sensitivity has ranged from 50% to 89%, and specificity from 29% to 89%. Second, there is disagreement on the definition of a positive test. How many of the different chromosomal abnormalities need to be seen in a cell, and how many cells need to be affected to score a sample as positive? Indeed, some laboratories have used criteria different

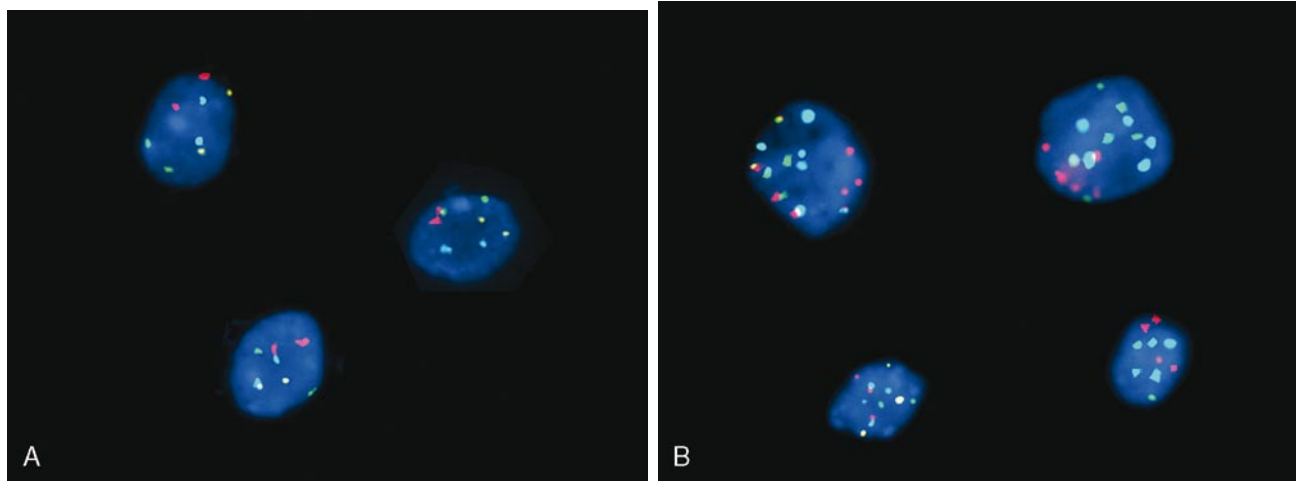


Figure 3.20 The UroVysion™ test for bladder cancer. A, Benign urothelial cell nuclei show two signals with fluorescent centromeric probes for chromosomes 3 (red), 7 (green), and 17 (aqua), and two intact segments of 9p21 with a fluorescent locus-specific probe (yellow). B, Malignant urothelial cells show increased copy number for chromosomes 3 (red), 7 (green), and 17 (aqua), and some nuclei show loss of 9p21 (yellow).

than those originally approved for use by the FDA.¹⁰³ Third, it is not clear whether the test is best ordered independently of cytology or as a reflex test in patients with negative or atypical cytology results. Finally, the ideal management of a patient with a positive FISH result but negative cytology and cystoscopy has not been established. In patients under surveillance for recurrent bladder cancer, a positive FISH result occurs in 26% of those with an otherwise negative workup.¹⁰³ Fifty percent to 80% of these patients will develop recurrent carcinoma within 29 months (their FISH result is thus considered an “anticipatory positive”), compared to 13% with negative FISH results. Thirty percent of patients presenting with hematuria have a positive FISH result but a negative urine cytology; 60% of these are later discovered to have UC. Despite laboratory variability, disagreement on the definition of a positive result, and the lack of a clear consensus on managing patients with discrepant results, the UroVysion™ FISH test can improve the detection of UC. The best way to integrate it into the workup of patients with hematuria or under surveillance for UC remains a challenge for urologists and cytologists.

SUMMARY

Urine and bladder washings—summary:

- Most urine specimens (for hematuria) are negative.
- The value of urine cytology for high-grade lesions is undisputed.
- The cytologic criteria for low-grade lesions (cytoplasmic homogeneity, high nuclear-to-cytoplasmic ratio, irregular nuclear outline) lack specificity.
- Clusters of urothelial cells per se are of limited use for the diagnosis of UC in voided urine.

- The term *dysplasia* should be avoided in cytologic specimens.
- Upper tract lesions should be diagnosed conservatively.
- Separating high-risk from low-risk patterns may be of value in reducing the number of atypical diagnoses.
- A commercially available FISH test (UroVysion™) is a promising adjunct to cytology in the detection of UC.

REFERENCES

1. Papanicolaou GN, Marshall VF: Urine sediment smears: a diagnostic procedure in cancers of the urinary tract. *Science* 1945;101:519-521.
2. Koss LG: *Diagnostic Cytology of the Urinary Tract*. Philadelphia, Lippincott-Raven, 1996.
3. Grossfeld GD, Litwin MS, Wolf JS Jr, et al.: Evaluation of asymptomatic microscopic hematuria in adults: the American Urological Association best practice policy—part II: patient evaluation, cytology, voided markers, imaging, cystoscopy, nephrology evaluation, and follow-up. *Urology* 2001; 57(4):604-610.
4. Hofland CA, Mariani AJ: Is cytology required for a hematuria evaluation? *J Urol* 2004;171(1):324-326.
5. Britton JP, Dowell AC, Whelan P, Harris CM: A community study of bladder cancer screening by the detection of occult urinary bleeding. *J Urol* 1992;148(3):788-790.
6. Messing EM, Madeb R, Young T, et al.: Long-term outcome of hematuria home screening for bladder cancer in men. *Cancer* 2006;107(9):2173-2179.
7. Mohr DN, Offord KP, Owen RA, Melton LJ 3rd: Asymptomatic microhematuria and urologic disease. A population-based study. *JAMA* 1986;256(2):224-229.
8. Gamarra MC, Zein T: Cytologic spectrum of bladder cancer. *Urology* 1984;23(3 Suppl):23-26.
9. Ellwein LB, Farrow GM, Friedell GH, Greenfield RE: An assessment of the impact of urine cytology screening using a computer-based model of bladder cancer. *Urol Clin N Am* 1984;11:585-598.

10. Holmquist ND: Detection of urinary tract cancer in urinalysis specimens in an outpatient population. *Am J Clin Pathol* 1988;89:499-504.
11. Crabbe JG, Cresdee WC, Scott TS, William MH: Cytologic diagnosis of bladder tumors amongst dye-stuff workers. *Br J Indust Med* 1956;13:270-276.
12. Crosby JH, Allsbrook WC, Koss LG, et al.: Cytologic detection of urothelial cancer and other abnormalities in a cohort of workers exposed to aromatic amines. *Acta Cytol* 1991;35:263-268.
13. De Ridder D, van Poppel H, Demonty L, et al.: Bladder cancer in patients with multiple sclerosis treated with cyclophosphamide. *J Urol* 1998;159(6):1881-1884.
14. Murphy WM: Current status of urinary cytology in the evaluation of bladder neoplasms. *Hum Pathol* 1990;21:886-896.
15. Rife CC, Farrow GM, Utz DC: Urine cytology of transitional cell neoplasms. *Urol Clin N Am* 1979;6:599-612.
16. Layfield LJ, Elsheikh TM, Fili A, et al.: Review of the state of the art and recommendations of the Papanicolaou Society of Cytopathology for urinary cytology procedures and reporting: The Papanicolaou Society of Cytopathology Practice Guidelines Task Force. *Diagn Cytopathol* 2004;30(1):24-30.
17. Morse N, Melamed MR: Differential counts of cell populations in urinary sediment smears from patients with primary epidermoid carcinoma of the bladder. *Acta Cytol* 1974;18:312-315.
18. Dodd LG, Johnston WW, Robertson CN, Layfield LJ: Endoscopic brush cytology of the upper urinary tract: Evaluation of its efficacy and potential limitations in diagnosis. *Acta Cytol* 1997;41:377-384.
19. Renshaw AA: Comparison of ureteral washings and biopsy specimens in the community setting. *Cancer Cytopathol* 2006;108:45-48.
20. Blute RB, Gittes RR, Gittes RF: Renal brush biopsy: survey of indications, techniques and results. *J Urol* 1981;126:146-149.
21. Chaubal A, McCue PA, Bagley D, Bibbo M: Multimodal cytologic evaluation of upper urinary tract urothelial lesions. *J Surg Pathol* 1995;1:31-35.
22. Gibod DB, Chiche R, Dalian D, Steg A: Upper tract urothelial tumors. Diagnostic efficiency of radiology and urinary cytology. *Eur Urol* 1982;8:145-147.
23. Potts SA, Thomas PA, Cohen MA, Raab SS: Diagnostic accuracy and key cytologic features of high grade transitional cell carcinoma in the upper urinary tract. *Mod Pathol* 1997;10:657-662.
24. Rubben H, Hering F, Dahm HH, Lutzeyer W: Value of exfoliative urinary cytology for differentiation between uric acid stone and tumor of upper urinary tract. *Urology* 1982;20:571-573.
25. Zincke H, Aguilo JJ, Farrow GM, et al.: Significance of urinary cytology in the early detection of transitional cell cancer of the upper urinary tract. *J Urol* 1976;116:781-783.
26. Constantian HM, De Girolami E: Urothelial tumors detected by cytology: New cell block technique. *J Urol* 1973;109(2):304-307.
27. Wolinska WH, Melamed MR: Urinary conduit cytology. *Cancer* 1973;32:1000-1006.
28. Schoones R, Gamarra MG, Moore GP: The diagnostic value of urinary cytology in patients with bladder carcinoma. *J Urol* 1971;106:693-696.
29. Kern WH: The grade and pathologic stage of bladder cancer. *Cancer* 1984;53:1185-1189.
30. Zein TA, Milad MF: Urine cytology in bladder tumors. *Int Surg* 1991;76:52-54.
31. Raab SS, Grzybicki DM, Vrbic CM, Geisinger KR: Urine cytology discrepancies: Frequency, causes, and outcomes. *Am J Clin Pathol* 2007;127(6):946-953.
32. Esposti PL, Zajicek J: Grading of transitional cell neoplasms of the urinary bladder from smears of bladder washings. *Acta Cytol* 1972;16:529-537.
33. Lewis RW, Jackson AC, Murphy WM, et al.: Cytology in the diagnosis and followup of transitional cell carcinoma of the urothelium: A review with a case series. *J Urol* 1976;116:43-46.
34. Koss LG, Deitch D, Ramanathan R, Sherman AB: Diagnostic value of cytology of voided urine. *Acta Cytol* 1985;29:810-816.
35. Wiener HG, Vooijs GP, van't Hof-Grootenboer B: Accuracy of urinary cytology in the diagnosis of primary and recurrent bladder cancer. *Acta Cytol* 1993;37:163-169.
36. Badalament RA, Hermansen DK, Kimmel M, et al.: The sensitivity of bladder wash flow cytometry, bladder wash cytology, and voided cytology in the detection of bladder carcinoma. *Cancer* 1987;60:1423-1427.
37. Foot NC, Papanicolaou GN, Holmquist ND, Seybolt JF: Exfoliative cytology of urinary sediments. *Cancer* 1958;11:127-137.
38. Park CH, Britsch C, Uson AC, Veenema RJ: Reliability of positive exfoliative cytologic study of the urine tract malignancy. *J Urol* 1969;102:91-92.
39. Bastacky S, Ibrahim S, Wilczynski SP, Murphy WM: The accuracy of urinary cytology in daily practice. *Cancer Cytopathol* 1999;87:118-128.
40. Halling KC, King W, Sokolova IA, et al.: A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. *J Urol* 2000;164:1768-1775.
41. Curry JL, Wojcik EM: The effects of the current World Health Organization/International Society of Urologic Pathologists bladder neoplasm classification system on urine cytology results. *Cancer* 2002;96(3):140-145.
42. Esposti PL, Moberger G, Zajicek J: The cytologic diagnosis of transitional cell tumors of the urinary bladder and its histologic basis. *Acta Cytol* 1970;14:145-155.
43. Loening S, Narayana A, Yoder L, et al.: Longitudinal study of bladder cancer with cytology and biopsy. *Br J Urol* 1978;50:496-501.
44. Umiker W: Accuracy of cytologic diagnosis of cancer of the urinary tract. *Acta Cytol* 1964;8:186-193.
45. Beyer-Boon ME, de Voogt HJ, van der Velde EA, et al.: The efficacy of urinary cytology in the detection of urothelial tumours. Sensitivity and specificity of urinary cytology. *Urol Res* 1978;6:3-12.
46. Boon L, Bianchini E, Altavilla G: Polyomavirus infection versus high grade bladder carcinoma. *Acta Cytol* 1989;33:887-893.
47. Murphy WM, Soloway MS, Jukkola AF, et al.: Urinary cytology and bladder cancer. The cellular features of transitional cell neoplasms. *Cancer* 1984;53:1555-1565.
48. Farrow GM: Urine cytology of transitional cell carcinoma: Diagnostic efficacy. In Weid GL, Keebler CM, Koss LG, et al. (eds): *Compendium on Diagnostic Cytology*, 7th ed. Chicago, 1992, pp. 302-306.
49. Farrow GM, Utz DC, Rife CC, Greene LF: Clinical observations on sixty nine cases of in situ carcinoma of the urinary bladder. *Cancer Res* 1977;37:2794-2798.
50. Matzkin H, Moinuddin SM, Soloway MS: Value of urine cytology versus bladder washings in bladder cancer. *Urology* 1992;38:711-719.
51. Murphy WM, Crabtree WM, Jukkola AF, Soloway MS: The diagnostic value of urine versus bladder washings in patients with bladder cancer. *J Urol* 1981;126:320-322.
52. Zein T, Wajzman Z, Englander LS, et al.: Evaluation of bladder washings and urine cytology in the diagnosis of bladder cancer and its correlation with selected biopsies of the bladder mucosa. *J Urol* 1984;132:670-671.
53. National Bladder Cancer Collaborative Group A: Cytology and histopathology of bladder cancer cases in a prospective longitudinal study. *Cancer Res* 1977;37:2911-2915.
54. Melamed MR, Wolinska WH: On the significance of intracytoplasmic inclusions in the urinary sediment. *Am J Pathol* 1961;38:711-719.

55. Renshaw AA, Madge R, Granter SR: Intracytoplasmic eosinophilic inclusions (Melamed-Wolinska bodies) are associated with metastatic transitional cell carcinoma in pleural fluid. *Acta Cytol* 1997;41:995-998.
56. Loo CK, Gune S: Trichomonas vaginalis in urine cytology. *Acta Cytol* 2000;44(3):484-485.
57. Niewiadomski S, Florentine BD, Cobb CJ: Cytologic identification of trichomonas vaginalis in urine from a male with long-standing sterile pyuria. *Acta Cytol* 1998;42(4):1060-1061.
58. Petrin D, Delgaty K, Bhatt R, Garber G: Clinical and microbiological aspects of Trichomonas vaginalis. *Clin Microbiol Rev* 1998;11(2):300-317.
59. Herawi M, Parwani AV, Chan T, et al.: Polyoma virus-associated cellular changes in the urine and bladder biopsy samples: A cytohistologic correlation. *Am J Surg Pathol* 2006;30(3):345-350.
60. Itoh S, Nakamura Y, Ohta Y, et al.: Cytologic and genetic study of polyomavirus-infected or polyomavirus-activated cells in human urine. *Arch Pathol Lab Med* 1998;122:333-337.
61. Del Mistro A, Koss LG, Braunstein J, et al.: Condyloma acuminata of the urinary bladder. *Am J Surg Pathol* 1988;12:205-215.
62. Koshikawa T, Leyh H, Schenck U: Difficulties in evaluating urinary specimens after local mitomycin therapy of bladder cancer. *Diagn Cytopathol* 1989;5(2):117-121.
63. Droller MJ, Erozan YS: Thiotepa effects on urinary cytology in the interpretation of transitional cell cancer. *J Urol* 1985;134:671-674.
64. Highman W, Wilson E: Urine cytology in patients with calculi. *J Clin Pathol* 1982;35:350-356.
65. Kannan V, Gupta D: Calculus artifact. A challenge in urinary cytology. *Acta Cytol* 1999;43(5):794-800.
66. Kroft SH, Oyasu R: Urinary bladder cancer: mechanisms of development and progression. *Lab Invest* 1994;71:158-173.
67. Murphy WM, Beckwith JB, Farrow GM: Tumors of the Kidney, Bladder, and Related Structures. In Rosai J (ed): *Atlas of Tumor Pathology*, 3rd ed.. Bethesda, Md, Armed Forces Institute of Pathology, 1994.
68. Jemal A, Siegel R, Ward E, et al.: Cancer statistics, 2007. *CA Cancer J Clin* 2007;57(1):43-66.
69. Epstein JI, Amin MB, Reuter VE, Mostofi FK: The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. *Am J Surg Pathol* 1999;22:1435-1448.
70. Raab S, Lenel J, Cohen M: Low grade transitional cell carcinoma of the bladder. Cytologic diagnosis by key features as identified by logistic regression analysis. *Cancer* 1994;74:1621-1626.
71. Renshaw AA, Nappi D, Weinberg DS: The cytology of grade 1 papillary transitional cell carcinoma: A comparison of cytologic, architectural, and morphometric criteria in cystoscopically obtained urine. *Acta Cytol* 1996;40:676-682.
72. El-Fakharany M, Mazzara P: Cytologic features of urothelial carcinoma in catheterized urine with cellular features. *Acta Cytol* 2006;50:312-316.
73. Goldstein ML, Whitman T, Renshaw AA: Significance of cell groups in voided urine. *Acta Cytol* 1998;42:290-294.
74. Harris MJ, Schwinn CP, Morrow JW, et al.: Exfoliative cytology of the urinary bladder irrigation specimens. *Acta Cytol* 1971;15:385-399.
75. Kannan V, Bose S: Low grade transitional cell carcinoma and instrument artifact. A challenge in urine cytology. *Acta Cytol* 1993;37:899-902.
76. Doria MI, Saint Martin G, Wang HH, et al.: Cytologic features of clear cell carcinoma of the urethra and urinary bladder. *Diagn Cytopathol* 1996;14:150-154.
77. Holmang S, Borghede G, Johansson SL: Primary small cell carcinoma of the bladder: A report of 25 cases. *J Urol* 1995;153:1820-1822.
78. Borghi L, Bianchini E, Altavilla G: Undifferentiated small-cell carcinoma of the urinary bladder. *Diagn Cytopathol* 1995;13:61-65.
79. van Hoeven KH, Artymshyn RL: Cytology of small cell carcinoma of the urinary bladder. *Diagn Cytopathol* 1996;14:2692-2697.
80. Piscioi F, Detassis C, Polla E, et al.: Cytologic presentation of renal adenocarcinoma in urinary sediment. *Acta Cytol* 1983;27:383-390.
81. Bardales RH, Pitman MB, Stanley MW, et al.: Urine cytology of primary and secondary urinary bladder adenocarcinoma. *Cancer* 1998;84(6):335-343.
82. Krishnan B, Truong LD: Prostatic adenocarcinoma diagnosed by urinary cytology. *Am J Clin Pathol* 2000;113:29-34.
83. Deshpande V, McKee GT: Analysis of atypical urine cytology in a tertiary care center. *Cancer* 2005;105:468-475.
84. Renshaw AA: Subclassifying atypical urinary cytology specimens. *Cancer Cytopathol* 2000;90:222-229.
85. De May RM: *The Art and Science of Cytopathology*. Chicago, ASCP Press, 1996.
86. Aprikian AG, Sarkis AS, Reuter VE, et al.: Biological markers of prognosis in transitional cell carcinoma of the bladder: Current concepts. *Semin Urol* 1993;11:137-144.
87. Ross JS, Cohen MB: Ancillary methods for the detection of recurrent urothelial neoplasia. *Cancer Cytopathol* 2000;90:75-85.
88. Messing EM, Teot L, Korman H, et al.: Performance of urine test in patients monitored for recurrence of bladder cancer: A multicentric study in the United States. *J Urol* 2005;174:1238-1241.
89. Dalbagni G, Presti J, Reuter VE, et al.: Genetic alterations in bladder cancer. *Lancet* 1993;342:469-471.
90. Hopman AHN, Moesker O, Smeets AWGB, et al.: Numerical chromosome 1, 7, 9, and 11 aberrations in bladder cancer detected by in situ hybridization. *Cancer Res* 1991;51:644-651.
91. Poddighe PJ, Ramaekers FCS, Smeets AWGB, et al.: Structural chromosome 1 aberrations in transitional cell carcinoma of the bladder: Interphase cytogenetics combining a centromeric, telomeric, and library DNA probe. *Cancer Res* 1992;52:4929-4934.
92. Sandberg AA: Chromosome changes in bladder cancer: clinical and other correlations. *Cancer Genet Cytogenet* 1986;19:163-175.
93. Spruck CH, Ohneseit PE, Gonzalez-Zulueta M, et al.: Two molecular pathways to transitional cell carcinoma of the bladder. *Cancer Res* 1994;54:784-788.
94. Halling KC: Vysis UroVysion for the detection of urothelial carcinoma. *Expert Rev Mol Diagn* 2003;3:507-519.
95. Bubendorf L, Grilli B, Sauter G, et al.: Multiprobe FISH for enhanced detection of bladder cancer in voided urine specimens and bladder washings. *Am J Clin Pathol* 2001;116:79-86.
96. Friedrich MG, Toma MI, Hellstern A, et al.: Comparison of multitarget fluorescence in situ hybridization in urine with other noninvasive tests for detecting bladder cancer. *BJU Int* 2003;92:911-914.
97. Halling KC, King W, Sokolova IA, et al.: A comparison of BTA Stat, hemoglobin dipstick, telomerase and Vysis UroVysion assays for the detection of urothelial carcinoma in urine. *J Urol* 2002;167:2001-2006.
98. Marin-Aguilera M, Mengual L, Ribal MJ, et al.: Utility of fluorescence in situ hybridization as a non-invasive technique in the diagnosis of upper urinary tract urothelial carcinoma. *Eur Urol* 2007;51.

99. Skacel M, Fahmy M, Brainard JA, et al.: Multitarget fluorescence in situ hybridization assay detects transitional cell carcinoma in the majority of patients with bladder cancer and atypical or negative urine cytology. *J Urol* 2003;169:2101-2105.
100. Skacel M, Pettay JD, Tsiftsakakis EK, et al.: Validation of multicolor interphase fluorescence in situ hybridization assay for detection of transitional cell carcinoma on fresh and archival thin-layer, liquid based cytology slides. *Analyt Quant Cytol Histol* 2001;23:381-387.
101. Veeramachaneni R, Nordberg ML, Shi R, et al.: Evaluation of fluorescence in situ hybridization as an ancillary tool to urine cytology in diagnosing urothelial carcinoma. *Diagn Cytopathol* 2003;28:301-307.
102. Zellweger T, Benz G, Cathomas G, et al.: Multi-target fluorescence in situ hybridization in bladder washings for prediction of recurrent bladder cancer. *Int J Cancer* 2006;119:1660-1665.
103. Yoder BJ, Skacel M, Hedgepeth R, et al.: Reflex UroVysion testing of bladder cancer surveillance patients with equivocal or negative urine cytology. *Am J Clin Pathol* 2007;127:295-301.

Pleural, Pericardial, and Peritoneal Fluids

EDMUND S. CIBAS

SPECIMEN COLLECTION, PREPARATION, AND REPORTING TERMINOLOGY

ACCURACY

BENIGN ELEMENTS

NON-NEOPLASTIC CONDITIONS

Acute Serositis
Eosinophilic Effusions
Lymphocytic Effusions
Rheumatoid Pleuritis
Lupus Pleuritis
Other Non-Neoplastic Conditions

MALIGNANT EFFUSIONS

Primary Tumors

Diffuse Malignant Mesothelioma

Primary Effusion Lymphoma

Metastatic Tumors

Adenocarcinoma

Squamous Cell Carcinoma

Small Cell Carcinoma

Melanoma

Non-Hodgkin Lymphoma

Hodgkin Lymphoma

Multiple Myeloma

Acute and Chronic Leukemias

Sarcomas

Germ Cell Tumors

The pleural, pericardial, and peritoneal cavities are lined by a single layer of flat mesothelial cells called the serosa. Normally, these cavities are collapsed and contain only a small amount of fluid, enough to lubricate the adjacent surfaces as they move over each other with respiration, heartbeats, and intestinal peristalsis. In disease states, a greater amount of fluid—an effusion—accumulates. Effusions are classified clinically as transudative or exudative. *Transudates* result from an imbalance of hydrostatic and oncotic pressures. Common causes are congestive heart failure (CHF), cirrhosis, and the nephrotic syndrome. Transudates have a low lactate dehydrogenase (LDH) and low total protein concentration. *Exudates* result from injury to the mesothelium, as occurs with malignancy, pneumonia, lupus, rheumatoid pleuritis, pulmonary infarction, or trauma. Exudates have a relatively high LDH and total protein concentration. The distinction is made by protein concentration measurements performed in the clinical laboratory. The distinction is important because pleural involvement by a malignancy causes an exudate, and therefore cytologic examination is not needed for a transudate.¹ Malignant tumors are a common cause of exudates because the serosal surfaces are a frequent site of metastasis for many tumors and the site of origin for the asbestos-related tumor malignant mesothelioma.

SPECIMEN COLLECTION, PREPARATION, AND REPORTING TERMINOLOGY

Specimens are obtained by inserting a needle into the pleural space (thoracentesis), pericardial space (pericardiocentesis), and peritoneal cavity (paracentesis). No more than 1500 mL of pleural fluid should be removed at any given time because larger evacuations can cause postexpansion pulmonary edema.¹ By contrast, large-volume paracentesis (e.g., 4 to 6 L) is relatively safe, and there are even reports of draining 20 L of ascites at one time.² Although peritoneal fluid is usually obtained through the abdominal wall, in women it can also be aspirated from the cul-de-sac through the vagina (culdocentesis). Less commonly, fluid is obtained by suction during thoracic, abdominal, or cardiac surgery.

Fluid is collected in clean containers and sent unfixed to the laboratory. To prevent clotting, which widely disperses cells, thus hindering their evaluation, fluid can be collected in heparinized bottles containing 3 units of heparin per milliliter of capacity.³ If heparinized bottles are not available, the heparin can be poured into the bottle before the fluid is collected; contact with glass results in rapid clotting.

Fluid is refrigerated at 4°C until the time of slide preparation. An effusion specimen is remarkably hardy—it can be refrigerated for 2 weeks or longer without compromising cellular morphology or antigenicity for immunostains.⁴ A variety of slide preparation methods are available. Slide preparation often begins by shaking the container to disperse the cells, then centrifuging a 50 mL aliquot (or the entire specimen if less than 50mL). The supernatant is discarded, and the sediment used to prepare direct smears, cytocentrifuge preparations,⁵ thinlayer slides^{5,6} or filter preparations. The slides are usually alcohol fixed and Papanicolaou stained. If a hematologic malignancy is suspected, air-dried cytocentrifuge preparations are helpful. One is stained with a Romanowsky-type stain and the rest can be reserved, if needed, for immunocytochemical studies for lymphocyte surface markers.⁷ So-called “cell blocks” are especially useful as adjuncts to the “cytologic” preparations previously listed. To prepare a cell block, the remainder of the sediment is wrapped in filter paper, placed in a cassette, embedded in paraffin, and cut and stained in the manner of histologic sections. Before placing it in a cassette, however, it is helpful to coagulate the sediment by adding a few drops of plasma and several drops of a thrombin solution.⁸⁻¹⁰ Clotting the specimen in this fashion does not disperse the diagnostic cells as does a spontaneously formed clot, but rather congeals the sediment into a compact mass. If the fluid was not heparinized and clots are present, they should be removed and placed in cassettes for processing as cell block material.

To improve sensitivity, most laboratories use two or more preparation methods for effusions. Smears alone identify about 67% of malignant fluids, whereas cytocentrifuge, filter, and cell block preparations detect 83% to 85%.¹¹ Cell block sections are especially useful for special stains and immunohistochemistry because of the ease with which multiple duplicate slides can be prepared, the relative absence of obscuring background staining, and the standardization of the preparation for control slides.¹² Cell blocks also make for excellent morphologic comparison with histopathologic sections (when, for example, the patient has had a prior breast biopsy) because they are fixed and stained in an identical manner.

In some laboratories, an unfixed wet smear stained with toluidine blue is prepared first to identify any fluid that contains large numbers of malignant cells. It is helpful to separate such fluids from the routine staining cycle to prevent cross-contamination.¹⁰

Leftover fluid is stored in the refrigerator in case additional slides are needed. Fresh fluid is sometimes useful for other studies, including flow cytometry,¹³⁻¹⁵ electron microscopy,¹⁶ and cytogenetic or molecular genetic analysis.^{17,18}

Most laboratories report results using descriptive terminology accompanied by simple primary headings such as “no malignant cells identified,” “suspicious cells present,” and “positive for malignant cells.” A case is called suspicious when the abnormal cells are too poorly preserved or too few to support a definite diagnosis of malignancy; this occurs in about 5% of specimens.^{19,20} In this situation, if the patient does have a malignancy involving the serosal cavity, fluid is likely to reaccumulate and the subsequent specimens may be diagnostic of malignancy. Criteria for the adequacy of an effusion specimen have not been established.

ACCURACY

Cytology is more sensitive than blind biopsy for detecting serosal malignancy (71% versus 45%),²¹ presumably because fluid provides a more representative sample. Estimates of the sensitivity of cytology for diagnosing serosal malignancy range from 58% to 71%.^{20,21} The cancer detection rate by cytology is increased by 2% to 38% when multiple specimens are examined.^{19,22,23} This still leaves a substantial false-negative rate. Thoracoscopy is the procedure of choice for patients with a strong clinical suspicion of pleural disease but a negative cytology result.²⁴

The specificity of a cytologic diagnosis is quite high; false-positive diagnoses occur in less than 1% of cases.^{19,25} When they occur, false-positive and false-suspicious diagnoses are caused by mesothelial cell atypia in the setting of pulmonary infarction,²¹ tuberculosis,¹⁹ chemotherapy,²⁶ acute pancreatitis,^{25,26} ovarian fibroma,¹⁹ and cirrhosis.²⁵ In children, false-positives result from misinterpreting benign lymphoid cells as lymphoma or neuroblastoma.²⁷

Immunocytochemistry is an essential adjunct to cytomorphology in selected cases and substantially improves diagnostic accuracy.

When to use immunohistochemistry for effusions:

- confirming malignancy when morphology alone is equivocal
- screening an effusion for lobular breast cancer
- distinguishing adenocarcinoma from mesothelioma
- establishing the primary site of a malignant effusion; a patient with:
 - an occult primary
 - multiple primaries

The circumstances outlined in the box represent common applications for immunohistochemistry. Antibodies against carcinoembryonic antigen (CEA),

B72.3, and a number of other markers have high sensitivity and specificity for malignancy and are extremely useful in a variety of settings, particularly for resolving cases that are cytologically equivocal.²⁸ Detailed discussion of specific applications is found in the sections that follow.

BENIGN ELEMENTS

Benign effusions contain mesothelial cells, histiocytes, and lymphocytes in varying proportions. Because some bleeding is common during specimen collection, red and white blood cells are common contaminants.

CYTOMORPHOLOGY OF MESOTHELIAL CELLS:

- often numerous
- dispersed as isolated cells
- occasional small clusters with “windows”
- round cells
- round nucleus
- single nucleolus
- dense cytoplasm with clear outer rim (“lacy skirt”)

Mesothelial cells can be sparse or numerous in benign effusions (Fig. 4.1). They are mainly dispersed as isolated cells or occasional small clusters. Large clusters composed of more than 12 cells are highly unusual in benign effusions. Binucleation and multinucleation are common, and mesothelial cells in mitosis can be seen in benign effusions. The dense cytoplasm reflects the abundance of tonofilaments, and the clear outer rim (“lacy skirt” or “halo”) corresponds to long, slender microvilli, better visualized with electron micros-

copy. Two or more mesothelial cells in groups are often separated by a narrow space or “window.” Less commonly, mesothelial cells have one or more cytoplasmic vacuoles.

With acute or chronic injury, mesothelial cells undergo hyperplasia and hypertrophy and can have significant nuclear atypia, but they remain predominantly dispersed as isolated cells. Such “reactive” mesothelial cells generally comprise a spectrum of cells that range from normal to “atypical,” with variation in nuclear size, a coarse chromatin texture, irregular nuclear contours, or prominent nucleoli (Fig. 4.2).

DIFFERENTIAL DIAGNOSIS OF REACTIVE MESOTHELIAL CELLS:

- mesothelioma
- metastatic malignancy

Malignant mesothelioma should be considered if there is marked atypia, particularly if the cells are much larger than normal, or if the fluid contains numerous clusters with 12 or more mesothelial cells, even if the cells themselves are not particularly atypical. Such large groups are uncommon in benign effusions. Clinical correlation is important because it may account for the atypia; some medical conditions, including anemia, cirrhosis, lupus, pulmonary infarction, renal failure, and AIDS,²⁹ are notorious causes of mesothelial atypia. On the other hand, if the patient has a large, unexplained, unilateral effusion, particularly with radiographic evidence of pleural thickening, additional evaluation (pleural biopsy, cytogenetics or molecular genetics) should be considered to exclude mesothelioma.^{17,18}

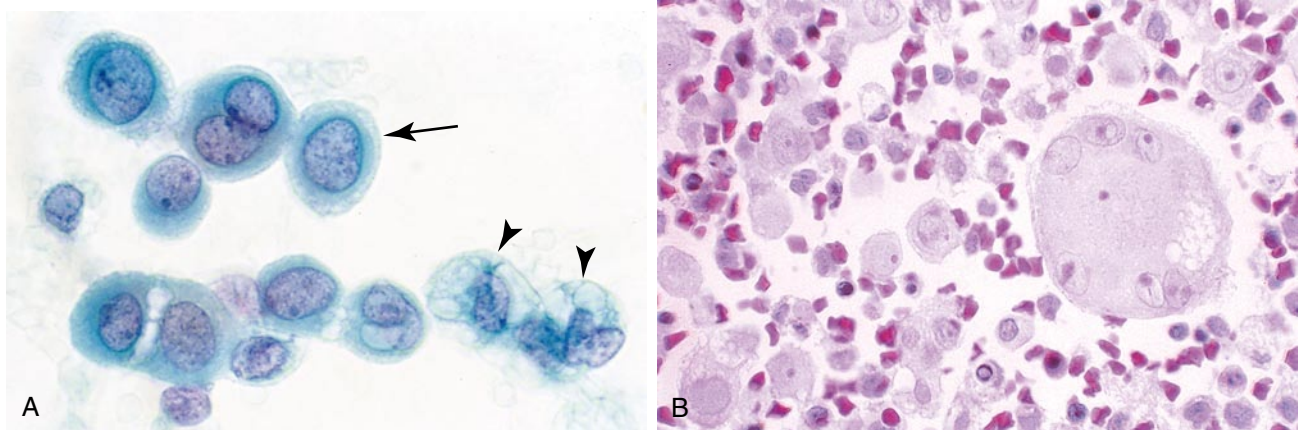


Figure 4.1 Mesothelial cells. A, Many characteristic features of mesothelial cells are seen here: the peripheral lucent zone, or “lacy skirt” (arrow); the dense perinuclear zone; the occasional binucleation; and the slitlike separation (“window”) between adjacent cells. A few histiocytes (arrowheads), with folded nuclei and vacuolated cytoplasm, are also present. B, Peripheral halos are well seen in this cell block section. Note also the large multinucleated mesothelial cell, a nonspecific finding.

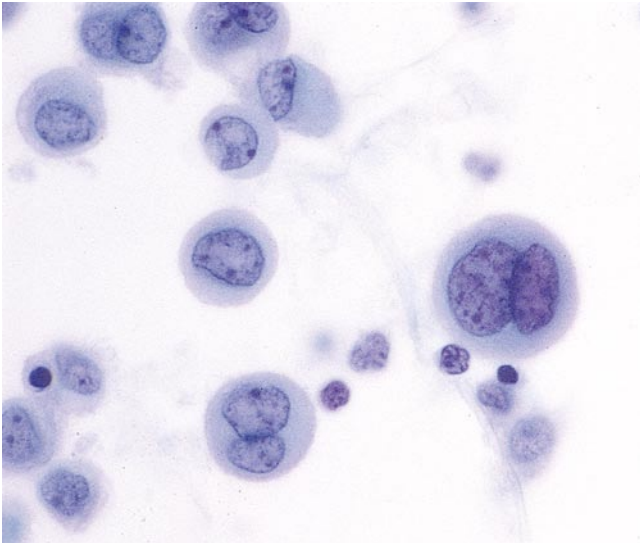


Figure 4.2 Reactive mesothelial atypia (peritoneal fluid, cirrhosis). Some benign fluids contain a population of moderately enlarged mesothelial cells with large, hyperchromatic, irregular nuclei.

Metastatic malignancy should be considered when a second population of cells is identified that is morphologically distinct from the mesothelial cells. In a minority of malignant effusions, a second population of cells is not evident. This is particularly true with lobular carcinoma of the breast and melanoma, the cells of which mimic normal histiocytes or mesothelial cells. Special stains are then needed to resolve the case.

CYTOMORPHOLOGY OF HISTIOCYTES:

- smaller nucleus than that of mesothelial cells
- nucleus often folded
- cytoplasm granular or vacuolated
- no “windows” between adjacent cells
- dense aggregates (cell block sections)

Some effusions contain abundant histiocytes (Fig. 4.3A). A particularly marked histiocytic reaction to irritation of the serosal surfaces has been termed *nodular histiocytic hyperplasia*.^{30,31} This is a nonspecific chronic inflammatory reaction that should not be misconstrued as a malignancy in cytologic or histologic specimens.^{10,31} When abundant, histiocytes can form aggregates on smears and liquid-based preparations,³¹ and they tend to sediment together in cell block preparations, forming mass-like aggregates that mimic malignancy. Immunohistochemistry can be useful to distinguish histiocytes from mesothelial cells and metastatic carcinoma; histiocytes are immunoreactive for CD68 and negative for keratin proteins (Fig. 4.3B); the reverse is true for mesothelial cells and metastatic carcinoma.

NON-NEOPLASTIC CONDITIONS

In many benign disorders, effusions give a nonspecific cytologic picture. Thus, pleural fluid in congestive heart failure or pulmonary infarction is morphologically indistinguishable from pericardial fluid caused by renal failure and peritoneal fluid as a result of cirrhosis. Fortunately, the features of some benign conditions are sufficiently characteristic to narrow the differential diagnoses or even indicate the specific etiology. To give an unusual example, finding undigested meat and vegetable matter in pleural fluid strongly suggests esophageal rupture (Boerhaave's syndrome).³²

Acute Serositis

Acute pleuritis, pericarditis, and peritonitis are usually the result of a bacterial infection. Bacterial infection of the pleura occurs in the setting of pneumonia, which secondarily involves the overlying pleura and results in a

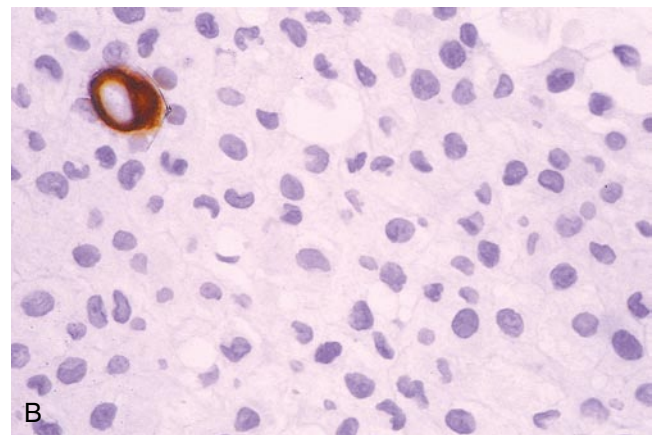
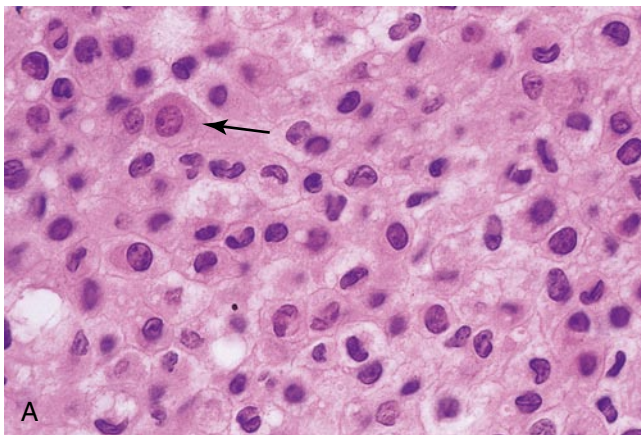


Figure 4.3 Histiocytes (pleural fluid, congestive heart failure). A, Like mesothelial cells, histiocytes are usually dispersed as isolated cells, but centrifugation for cell block sections compresses them into large groups. Compare the histiocytes, which have an oval or folded nucleus, to the mesothelial cell (arrow). B, Only the mesothelial cell is immunoreactive for cytokeratins.

pleural empyema. Acute infection of the peritoneal cavity is often secondary to inflammation of or injury to the bowel, as in spontaneous bacterial peritonitis.

The fluid is a creamy pale yellow (purulent) and often foul-smelling. Cytologic preparations are highly cellular and composed almost exclusively of polymorphonuclear leukocytes. Bacteria are demonstrated with special stains in some cases.

It is important to screen such cases carefully for malignant cells because acute infection can be a complication of metastatic malignancy.

Eosinophilic Effusions

A pleural effusion is considered “eosinophilic” when eosinophils account for 10% or more of the nucleated cells present. Between 5% and 16% of exudative effusions are eosinophilic effusions.³³ The most common causes are pneumothorax and hemothorax.²⁴ The introduction of air or blood into the pleural space, so often the reason behind an eosinophilic effusion, can occur simply with repeated thoracenteses. Less common causes include drug reactions, parasitic infections, pulmonary infarction, and the Churg-Strauss syndrome.^{24,34} In about one third of cases the origin remains obscure.³⁵ Most cases resolve spontaneously.

Eosinophilic pericardial and peritoneal effusions are less common than eosinophilic pleural effusions.

Cytologic preparations are usually cellular and remarkable for a high concentration of eosinophils. On alcohol-fixed Papanicolaou-stained slides, the defining eosinophilic cytoplasmic granules are either orangeophilic or pale-green and inconspicuous, and the cells are identified more on the basis of their bilobed nuclei (Fig. 4.4). The granules are brightly eosinophilic on cell block preparations stained with hematoxylin and eosin (H & E), however, and on air-dried Romanowsky-

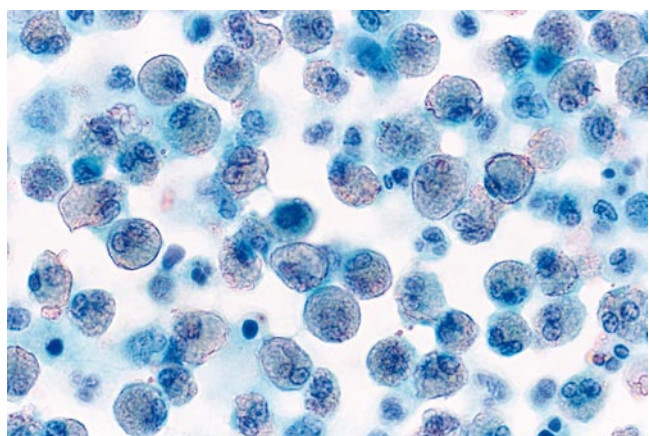


Figure 4.4 Eosinophilic pleural effusion. Numerous eosinophils in pleural fluid are more commonly associated with benign conditions, like a pneumothorax (as in this case) or hemothorax.

stained slides. Charcot-Leyden crystals (see Fig. 2.10) are present in some cases, and curiously, are more common in fluids that have been refrigerated for more than 24 hours.³⁶

Lymphocytic Effusions

A pleural effusion consisting mostly of small lymphocytes is a relatively common but nonspecific finding. Cytologic preparations are often highly cellular and composed almost exclusively of dispersed, small lymphocytes.³⁷ Mesothelial cells and histiocytes are either conspicuously absent or present in small numbers.

DIFFERENTIAL DIAGNOSIS OF LYMPHOCYTIC EFFUSIONS:

- malignancy
- tuberculosis
- status post coronary artery bypass

Despite the absence of malignant cells, a malignancy is a common cause of a lymphocytic effusion. The malignancy may be nearby (e.g., in the lung) and may be obstructing lymphatic outflow but may not have spread to the pleural surfaces. Alternatively, a pleural malignancy may be evoking a peritumoral lymphocytic response, but the tumor itself is not shedding cells into the effusion.³⁰ It is not uncommon for the initial pleural fluids in patients with a pleural mesothelioma to consist only of lymphocytes.³⁸ Effusions caused by small lymphocytic lymphoma and chronic lymphocytic leukemia are quite uncommon. Because these are B-cell neoplasms, immunocytochemical or flow cytometric evaluation of lymphocyte surface markers is helpful in confirming the diagnosis. In a patient with chronic lymphocytic leukemia and a peripheral lymphocytosis, however, contamination of the effusion by peripheral blood during a traumatic tap should be excluded before diagnosing pleural involvement. Even a small amount of blood containing leukemic cells can result in a false-positive diagnosis. The diagnosis of tuberculosis can be confirmed by microbiologic studies or pleural biopsy, which reveals caseating granulomas and acid-fast organisms. The differential diagnosis includes other benign effusions of nontuberculous origin, as in patients after coronary artery bypass surgery.²⁴

Rheumatoid Pleuritis

Less than 5% of patients with rheumatoid arthritis develop pleural involvement by the same necrotizing granulomatous inflammation that causes joint disease. In almost all cases, joint disease precedes

the development of pleuritis, but occasionally pleuritis precedes or is synchronous with the onset of joint disease.^{39,40} The pleural effusion can be unilateral or bilateral, and some patients have a synchronous pericardial effusion. Radiographic studies reveal pulmonary nodules in a minority of patients; presumably, these are rheumatoid nodules. The effusion can last for days, months, or sometimes years.

The cytologic picture is so characteristic that it has been termed pathognomonic.³⁹ Examination of pleural fluid, therefore, can be extremely useful to confirm the diagnosis of rheumatoid pleuritis and exclude the possibility of coincident disease, especially a malignancy.

CYTOMORPHOLOGY OF RHEUMATOID PLEURITIS:

- abundant clumps of granular debris
- macrophages

Cytologic preparations are sparsely or moderately cellular. An abundant granular material dominates the picture (Fig. 4.5A). It can stain green, pink, red, or orange with the Papanicolaou stain, and aggregates into small and large clumps with irregular edges. Large, island-like masses can be appreciated in cell block material. The predominant cell is the macrophage, which is round or spindle-shaped; multinucleated macrophages are seen in most but not all cases (Fig. 4.5A and B). Lymphocytes and polymorphonuclear leukocytes may be seen. Mesothelial cells are noticeably absent.

The characteristic granular debris is different from fibrin, which is usually strandlike rather than coarsely granular. Although the elongated macrophages resemble the spindle cells seen in squamous and other cancers, their nuclei are normochromatic.

Lupus Pleuritis

About one third of patients with systemic lupus erythematosus (SLE) develop a pleural or pericardial effusion. Peritoneal effusions are less common but do occur. Rarely, an effusion is the initial manifestation.

The characteristic cell is the lupus erythematosus (LE) cell, a neutrophil or macrophage that contains an ingested cytoplasmic particle called a *hematoxylin body*. The hematoxylin body may be green, blue, or purple with the Papanicolaou stain, and magenta with Romanowsky-type stains, and has a glassy, homogeneous appearance (Fig. 4.6). Filling the cytoplasm of the neutrophil or macrophage, it often pushes the nucleus to one side, indenting it into a crescent-like shape. Hematoxylin bodies are thought to represent degenerated nuclei. Similar cells that contain ingested nuclei with a visible chromatin structure (rather than the glassy, structureless hematoxylin body) are called *tart cells* after the patient in whom they were first described.

Lupus erythematosus cells are present in just 27% of effusions in patients with systemic lupus erythematosus, and only in those with a known diagnosis of systemic lupus erythematosus.⁴¹

Other Non-Neoplastic Conditions

Most viral pneumonias associated with a pleural effusion result in a nonspecific cytologic picture. The cytopathic changes characteristic of the herpes viruses and cytomegalovirus (CMV) are rarely seen in serous effusions. Although fungal infections are common in patients who are immunocompromised, organisms are rarely seen in pleural, pericardial, and peritoneal fluids. *Candida* species, *Cryptococcus neoformans*, *Coccidioides immitis*, *Blastomyces dermatitidis*, and *Aspergillus niger* have been described in fluids in rare instances.¹⁰

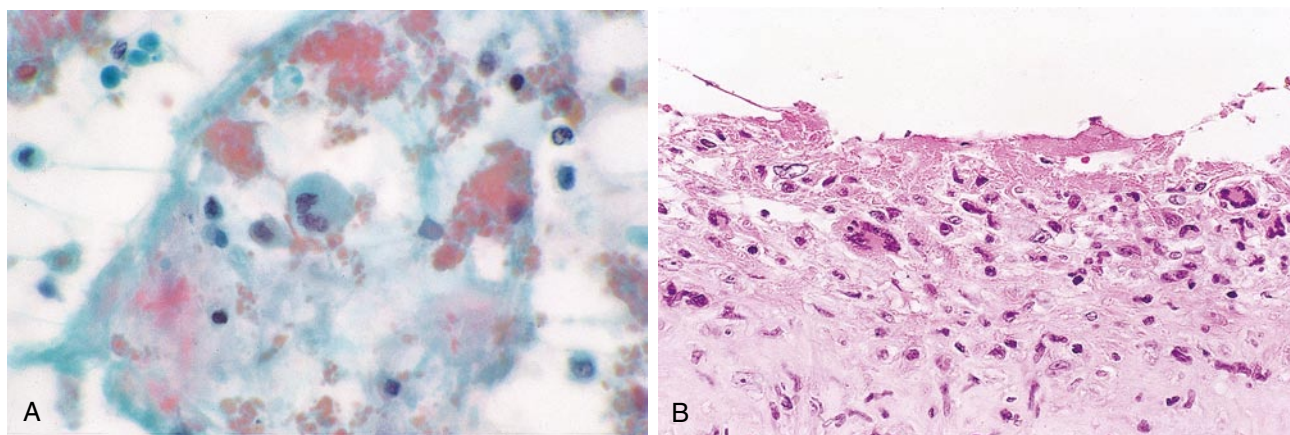


Figure 4.5 Rheumatoid pleuritis. A, Scattered multinucleated histiocytes and clumped granular debris in the background are characteristic of pleural fluids in patients with rheumatoid pleuritis. B, A pleural biopsy has the appearance of an “opened out” rheumatoid nodule, with epithelioid histiocytes, giant cells, and fibrinoid debris lining the pleural space.

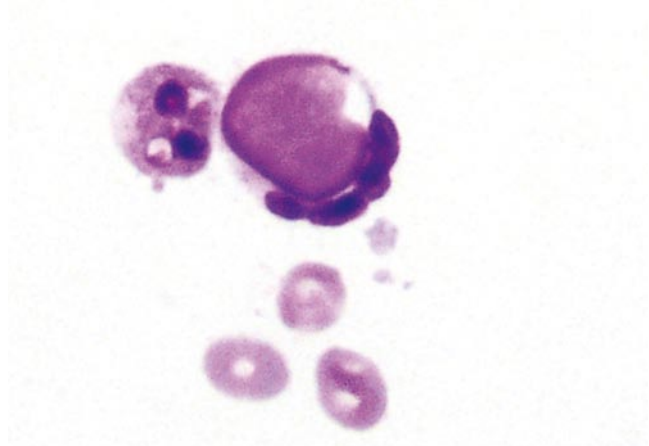


Figure 4.6 Hematoxylin body (lupus pleuritis). The lobes of the nucleus are pushed against the side of the neutrophil by a large, homogeneous, intracytoplasmic body (Wright-Giemsa).

Pneumocystis carinii has been identified in pleural and peritoneal effusions from patients who are immunocompromised.⁴²⁻⁴⁴ Papanicolaou stains show foamy exudates similar to those seen in respiratory specimens. The trophozoites measure 2.5 to 5.0 μm and have pale cytoplasm and a dot-like nucleus; they may be intracellular (within macrophages) or extracellular, and are well seen on air-dried preparations stained with a Romanowsky-type stain. Cyst forms measure 4 to 7 μm and can be seen with special stains like the methenamine silver stain.

MALIGNANT EFFUSIONS

Some tumors have a greater tendency than others to spread to the pleura, pericardium, or peritoneum. The most common are listed in Table 4.1. In children, the most common cause of a malignant pleural or peritoneal effusion is non-Hodgkin lymphoma.²⁷

Most patients with a malignant effusion have a previously documented primary neoplasm. In some cases, however, a malignant effusion is the first manifestation of an occult malignancy. The most common occult primary in women and men who present with a malignant pleural effusion is lung cancer. It is extremely uncommon for breast cancer to manifest itself initially as a malignant effusion.^{23,45,46} The most common occult sources of a malignant peritoneal effusion are intestinal and pancreatic cancer in men and ovarian cancer in women.^{46,47} Other tumors that can present as a malignant effusion include lymphoma, melanoma, and mesothelioma.⁴⁶ In some patients, the primary site is never discovered.^{23,46}

Malignant cells in pleural, pericardial, or peritoneal fluid betoken a grim prognosis. The median survival for patients with a positive pleural or peritoneal effusion

TABLE 4.1 THE MOST COMMON TUMORS THAT CAUSE MALIGNANT EFFUSIONS BY SITE AND SEX*

Site	Men	Women
Pleural	lung	breast
	lymphoma or leukemia	lung
	gastrointestinal tract	lymphoma or leukemia
	sarcoma	ovary
	mesothelioma	gastrointestinal tract
	genitourinary (kidney, prostate, bladder)	endometrium
	melanoma	sarcoma
		mesothelioma
Peritoneal	lymphoma or leukemia	ovary
	gastrointestinal tract	breast
	pancreas	endometrium
	lung	stomach
	sarcoma	lymphoma or leukemia
	prostate	colon and rectum
	melanoma	pancreas
	germ cell tumors	mesothelioma
	mesothelioma	

*Data from Sears D, Hajdu SI: The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. *Acta Cytol* 1987;31:85-97; Johnston WW: The malignant pleural effusion: A review of cytopathologic diagnoses of 584 specimens from 472 consecutive patients. *Cancer* 1985;56:905-909.

is less than 6 months.⁴⁸ Certain tumors, like estrogen-positive breast cancers and well-differentiated mucinous adenocarcinomas of the appendix, have a slightly better prognosis.

Systemic chemotherapy fails to alleviate most recurrent malignant effusions, with a few notable exceptions (e.g., those caused by lymphoma and small cell lung cancer). Because most malignant pleural effusions recur and impede respiration, chest tube placement or pleurodesis (sclerosis of the pleural cavity by injecting talc, doxycycline, or bleomycin) is often performed as a palliative measure.² For patients with recurrent malignant ascites, palliative treatment may consist of either repeated paracenteses, intraperitoneal chemotherapy, placing a drainage catheter, or implanting a peritoneovenous shunt (usually into the superior vena cava).^{2,48} Surprisingly, there is no evidence that disseminating tumor cells via a peritoneovenous shunt decreases survival.² The various treatment options have their advantages and disadvantages; selecting the best treatment option focuses on the patient's desires and improving the quality of life.

Tips for detecting malignant cells in effusions:

- "second population"
- numerous large clusters
- lacunae (cell block sections)

A good way to identify malignant cells in effusions is to first locate some benign mesothelial cells. With these as a benchmark, one searches for a “second population” of cells (not counting, of course, any lymphocytes or histiocytes) that is clearly different. Malignant cells are not necessarily larger than the mesothelial cells. Some are about the same size but are recognized because of their high nuclear-to-cytoplasmic ratio, nuclear hyperchromasia, or macronucleoli. Exceptions to this rule occur, notably mesothelioma, for which a sharp distinction between benign and neoplastic mesothelial cells is not appreciated.

Normal mesothelial cells virtually never form large cell clusters. Effusions with numerous large cell aggregates are easily spotted as malignant. Care must be taken not to confuse loosely clustered cells, which are a common artifact of cytocentrifugation and liquid-based preparations. Reliably malignant clusters are tightly cohesive.

Malignant cells in cell block sections are frequently situated in lacunae (Fig. 4.7). These clear spaces surrounding individual cells or groups of cells are seen in 75% of cell blocks of malignant effusions, mostly adenocarcinomas, but the finding is not specific; lacunae are also seen in one third of benign effusions.⁴⁹ Lacunae are helpful in locating suspicious cells at low magnification, but inspection at high magnification is needed for definitive diagnosis.

Primary Tumors

Primary tumors of the serosal surfaces are uncommon, being far outnumbered by secondary involvement by tumors from other locations. The two primary serosal malignancies considered here are malignant mesothelioma and primary effusion lymphoma.

Diffuse Malignant Mesothelioma

Diffuse malignant mesothelioma (for simplicity, referred to as *mesothelioma*) accounts for less than 2% of malig-

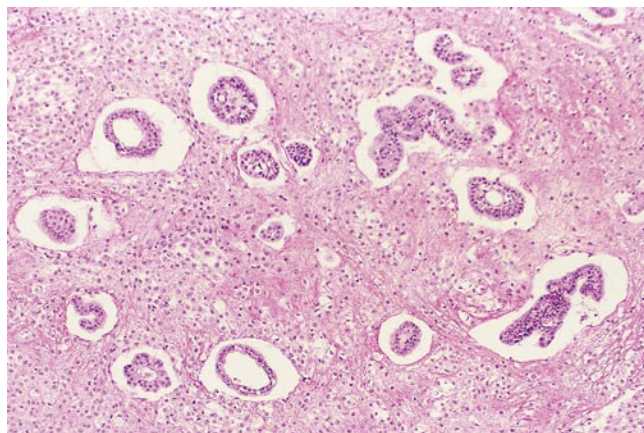


Figure 4.7 Cell block lacunae (pleural fluid). In cell block sections, malignant cells are often situated in an empty space (lacuna); the reason for this artifact is unknown. It is commonly seen with adenocarcinomas, rarely with lymphomas and melanoma.

nant effusions.^{30,50} Strongly linked in most cases to asbestos exposure, it arises most commonly in the pleura and less commonly in the peritoneum; primary tumors of the pericardium or tunica vaginalis of the testis are rare. The latency (time from first asbestos exposure to clinical disease presentation) is extremely long, with an average of 30 to 40 years. The peak incidence in the United States appears to have happened in the 1990s, but cases are still increasing in other countries like Great Britain and Australia.³⁰

Malignant mesothelial cells grow as multiple plaques that coalesce into larger nodules visible radiographically as a thickening of the pleura. Histologically, these tumors are classified as epithelial, sarcomatoid, or mixed (biphasic) types. The epithelial type comes in a dizzying variety of histologic patterns: epithelioid, deciduoid, tubulopapillary, microcystic, small cell, desmoplastic, and high grade (pleomorphic), but most of these variants are rare.³⁰ The most common patterns of epithelial mesothelioma are the epithelioid and tubulopapillary. Most mesotheliomas are, in fact, well-differentiated tumors and cytologically remarkably, deceptively bland. Mesotheliomas, like benign mesothelial cells, are immunoreactive for cytokeratins (including CK 5 and 6), desmin, vimentin, calretinin, and Wilms tumor protein 1 (WT1).

Common symptoms are chest pain and shortness of breath. Establishing the diagnosis is not always straightforward, with a median time from the onset of symptoms to diagnosis of 8 weeks.³⁸ Most patients have an effusion, usually unilateral, at the time of presentation, and the fluid is often described as having the color and consistency of honey. When suspicious and positive results are combined, the sensitivity of effusion cytology for the diagnosis of mesothelioma is only 32%.³⁸

CYTOMORPHOLOGY OF MESOTHELIOMA:

- two principal patterns:
 - large clusters with scalloped (“knobby”) edges
 - “noncohesive” (isolated cells)
- cytomegaly
- round, centrally placed nucleus
- prominent nucleolus
- binucleation and multinucleation
- dense cytoplasm with peripheral “halo”
- normal nuclear-to-cytoplasmic ratio
- windows

Only the epithelial and mixed (biphasic) types of mesothelioma are likely to exfoliate malignant cells; the pure sarcomatoid type rarely exfoliates. When the malignant cells exfoliate, the most common cytologic pattern is of numerous large clusters (morulae; Fig. 4.8A). The clusters are composed of up to hundreds

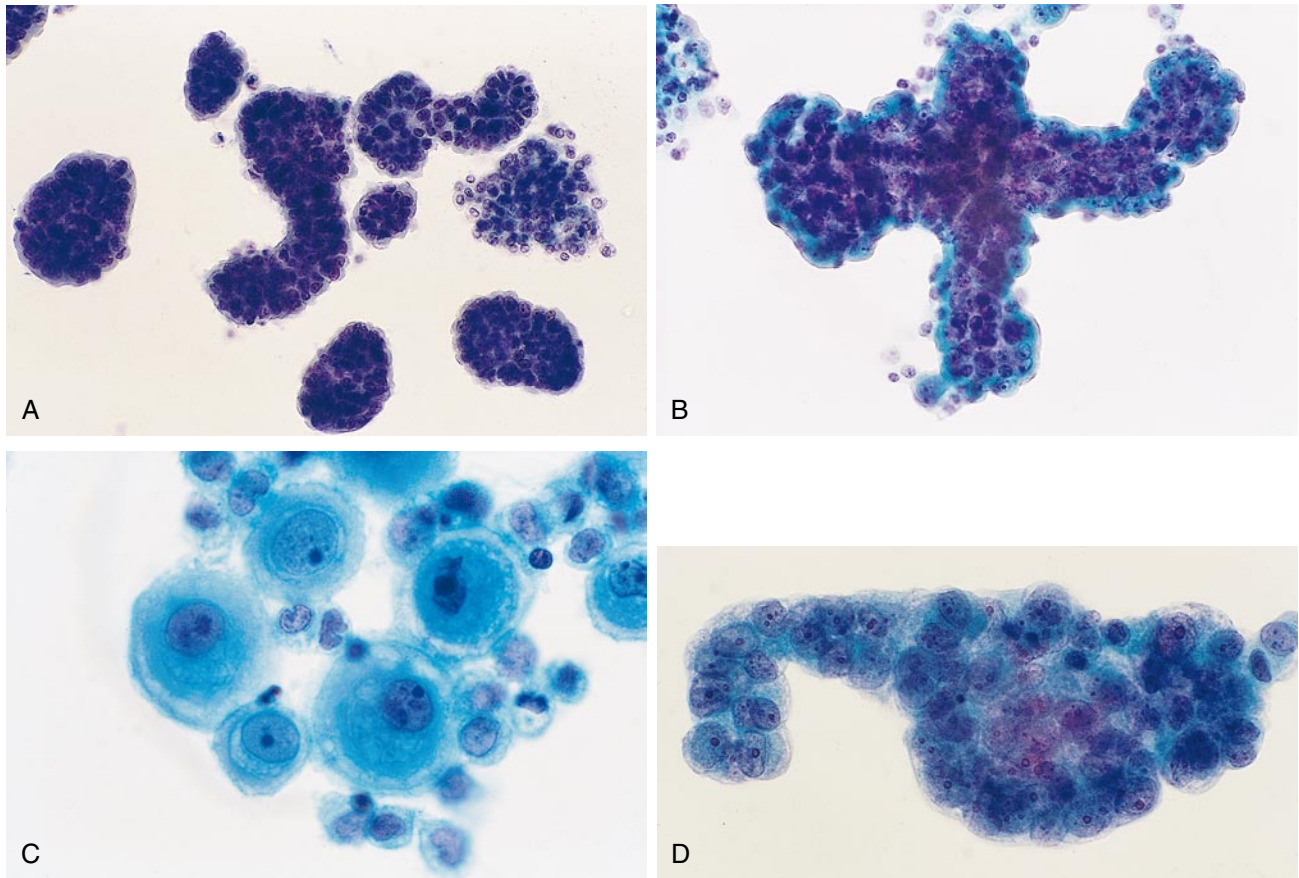


Figure 4.8 Malignant mesothelioma (pleural fluid). A., Solid, morulae-like spheres, some of them elongated, are composed of cells that resemble normal mesothelial cells. A fluid composed of many large clusters is virtually always malignant. B, A branching pattern is seen in some cases. Note the knobby contours. C, In most mesotheliomas, the nuclear-to-cytoplasmic ratio of normal mesothelial cells is recapitulated. D, In other cases, the nuclear-to-cytoplasmic ratio is significantly increased.

of cells that are recognizably mesothelial in origin, with round nuclei, prominent nucleoli, and dense cytoplasm with a pale rim. The morulae have a knobby contour (“mulberry” clusters), and some show branching (Fig. 4.8B). In most cases, the malignant mesothelial cells are larger than normal mesothelial cells, sometimes markedly so. Cytoplasm is abundant in most cases, and therefore the nuclear-to-cytoplasmic ratio is often deceptively normal (Fig. 4.8C), but cytoplasm can be scant in some cases (Fig. 4.8D). Occasionally, microvilli can be appreciated (Fig. 4.9). Nuclear atypia is mild in most cases. On cell block sections, the clusters are a solid mass of cells, or they may contain a collagenous or acid mucopolysaccharide core (Fig. 4.10).

Not all fluids have the mulberry-cluster pattern. In another rather common pattern, the malignant cells are not cohesive but instead are dispersed as isolated cells (see Fig. 4.8C).⁵¹ More unusual variants include tumors composed predominantly of vacuolated cells (Figs 4.11A and B), tumors that show small cell differentiation, those with an abundant lymphohistiocytic infiltrate, and those accompanied by psammoma bodies.⁵²

DIFFERENTIAL DIAGNOSIS OF MESOTHELIOMA:

- reactive mesothelial cells
- metastatic tumor
 - adenocarcinoma
 - squamous cell carcinoma
 - epithelioid hemangioendothelioma

Mesothelioma versus Reactive Mesothelial Cells. Because reactive mesothelial cells of the pleura and peritoneum do not form numerous large morulae, the diagnosis of mesothelioma is straightforward when the specimen is highly cellular and contains many large clusters of enlarged mesothelial cells (see Figs. 4.8A). Such a specimen can be called, at the very least, *suspicious for malignancy*. In the appropriate clinical context (unilateral effusion, asbestos exposure, and pleural thickening) it can be argued that they can reliably be called *positive for malignancy*.

As mentioned previously, the striking morular pattern is not seen in all mesotheliomas. In such cases, the distinction from reactive mesothelial cells is more

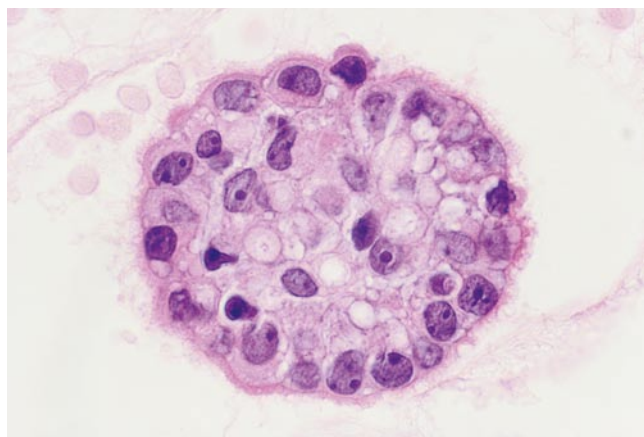


Figure 4.9 Malignant mesothelioma (pleural fluid). In some cases, the characteristic microvilli can be appreciated.

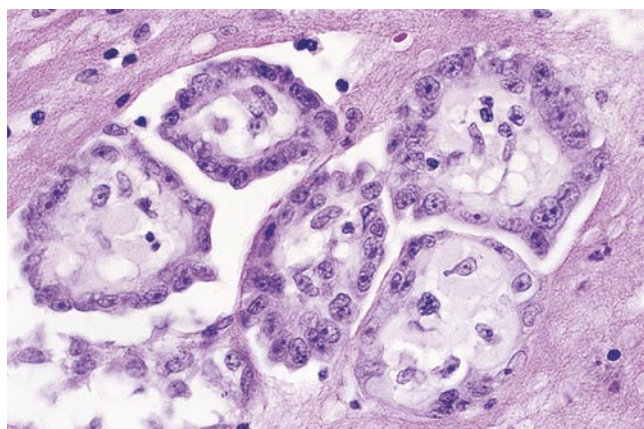


Figure 4.10 Malignant mesothelioma (pleural fluids). In some cases, cell block sections show that clusters of malignant cells surround a collagenous core.

problematic. There are precious few (if any) immunohistochemical markers on which one can rely for this distinction. The best marker for distinguishing benign from malignant mesothelial cells appears to

be epithelial membrane antigen (EMA), but not all investigators report good specificity. Although some find excellent specificity,⁵³⁻⁵⁶ especially for EMA clone E29,⁵³ others do not.^{57,58}

Although time consuming and not available in most laboratories, cytogenetic analysis has high sensitivity and specificity for the distinction between reactive mesothelial cells and mesothelioma. In almost all cases, mesotheliomas show clonal cytogenetic aberrations indicative of malignancy, the most common being deletions of 1p, 3p, 6q, 9p, and 22q.¹⁷ With a combination of appropriate probes, these deletions can be detected more easily by fluorescence in situ hybridization ([FISH]; Fig. 4.12).^{18,59}

Mesothelioma versus Adenocarcinoma. Some adenocarcinomas spread to involve the serosal surfaces in a diffusely infiltrative, “pseudomesotheliomatous” pattern that mimics mesothelioma. The similarity extends to histopathology and cytopathology—exfoliated cells of mesotheliomas and adenocarcinomas can be arranged in cohesive clusters or dispersed in a noncohesive pattern, and both can have vacuolated cytoplasm. A few morphologic clues exist, however. Mesothelioma cells form a morphologic continuum with benign-appearing mesothelial cells at one end, whereas fluids that harbor metastatic adenocarcinoma generally contain two distinct cell populations. Tumor cells that are separated by slitlike “windows” and have abundant, dense cytoplasm are more likely to be mesothelial in origin. On cell block sections, a core of edematous collagen and stromal cells, surrounded by neoplastic cells, is more commonly seen in mesothelioma than in adenocarcinoma (see Fig. 4.10). Conversely, ringlike structures with hollow cores, seen in some adenocarcinomas, are uncommon in mesothelioma. Clusters with a knobby (mulberry-like) contour, rather than the smooth, cannonball-like edge of many adenocarcinomas, are characteristic of mesotheliomas (see Fig. 4.8B).

These morphologic clues, unfortunately, are not always reliable. Exceptions occur frequently enough that,

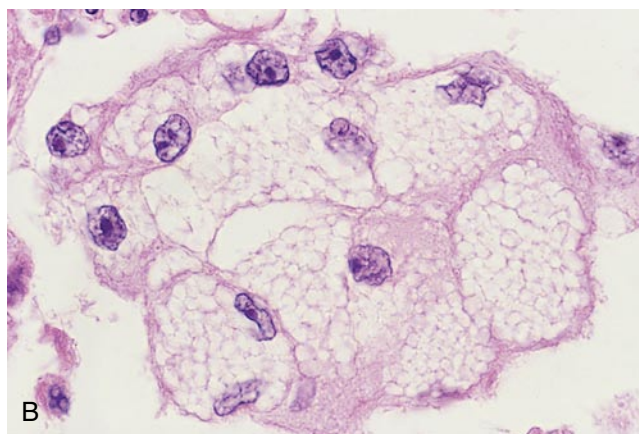
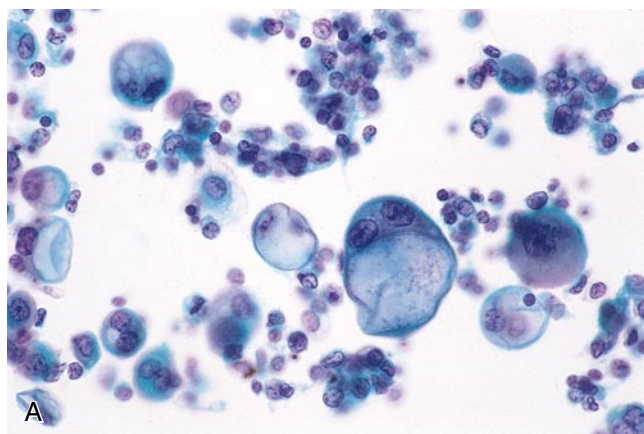


Figure 4.11 Malignant mesothelioma. A, B, Rarely, the tumor cells show striking cytoplasmic vacuolization.

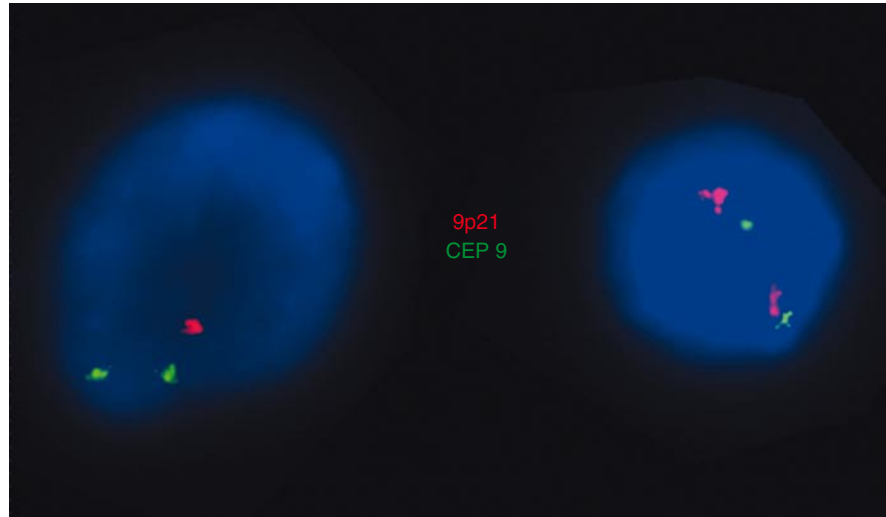


Figure 4.12 Malignant mesothelioma. Mesotheliomas are characterized genetically by clonal chromosomal deletions. Fluorescence in situ hybridization of pleural fluid shows a normal cell on the right and a mesothelioma cell on the left. The preparation has been incubated with probes for the centromeric region (green) and the deleted region (red) of chromosome 9. Both cells show two centromeric regions, but the mesothelioma cell is missing a segment of the short arm of chromosome 9. (Courtesy of Paola Dal Cin, Brigham and Women's Hospital, Boston.)

in a given case, morphology alone cannot be depended on for an unequivocal classification. Histochemical and immunocytochemical stains can make the distinction in almost all cases and are indispensable in this regard (Table 4.2). Two so-called mesothelial cell markers are particularly useful in this distinction. Calretinin, a calcium-binding protein, is strongly positive in most

mesotheliomas, demonstrating a nuclear and cytoplasmic staining pattern (Fig. 4.13A). By contrast, only a small number of adenocarcinomas are positive, virtually always in a predominantly cytoplasmic pattern.^{60,61} Similarly, the WT1 protein, the product of the WT1 gene, located on chromosome 11p and implicated in the pathogenesis of Wilms tumors and mesotheliomas, is strongly expressed in most mesotheliomas.^{62,63} The staining is nuclear, not cytoplasmic (Fig. 4.13B). With the exception of serous carcinomas, most adenocarcinomas are negative for WT1.

Mesotheliomas are typically negative for intracytoplasmic mucin with the mucicarmine and periodic acid-Schiff diastase (PAS-D) stains and negative for the carcinoma markers CEA, MOC-31, Ber-EP4, Leu-M1 (CD15), and B72.3.⁶⁴⁻⁶⁶ In contrast, at least half of adenocarcinomas in effusions are positive for cytoplasmic mucin, and most are immunoreactive for one or more of the carcinoma markers CEA, MOC-31, Ber-EP4, Leu-M1, and B72.3 (Fig. 4.14A). Thyroid transcription factor-1 (TTF-1) is particularly useful in the frequent distinction between lung adenocarcinoma and mesothelioma. Strong nuclear staining is common in lung adenocarcinomas and absent in mesotheliomas (Fig. 4.14B).^{63,67}

A few caveats are in order. First, a critical analysis of the mucin stains is important. Fine cytoplasmic granules seen with the PAS-D stain, the interposition of positively stained basement membrane material, and an extracellular localization of staining should not be misinterpreted as a positive reaction for mucin.

TABLE 4.2 COMMON HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STAINING PATTERNS FOR MESOTHELIOMA AND METASTATIC ADENOCARCINOMA

Stain	Expected Result*	
	Adenocarcinoma	Mesothelioma
PAS-D	+	—
mucicarmine	+	—
CEA	+	—
MOC-31	+	—
Ber-EP4	+	—
Leu M-1	+	—
B72.3	+	—
TTF-1	+(nuclear) [†]	—
calretinin	—	+ (nuclear and cytoplasmic)
WT1	—	+ (nuclear) [‡]

CEA, carcinoembryonic antigen; PAS-D, periodic acid-Schiff and diastase;

TTF-1, thyroid transcription factor-1; WT1, Wilms tumor 1 protein.

*Result observed in most but not all cases

[†]Positive in adenocarcinomas of the lung and thyroid only. Also positive in small cell carcinomas.

[‡]Also positive in serous carcinomas of the ovary.

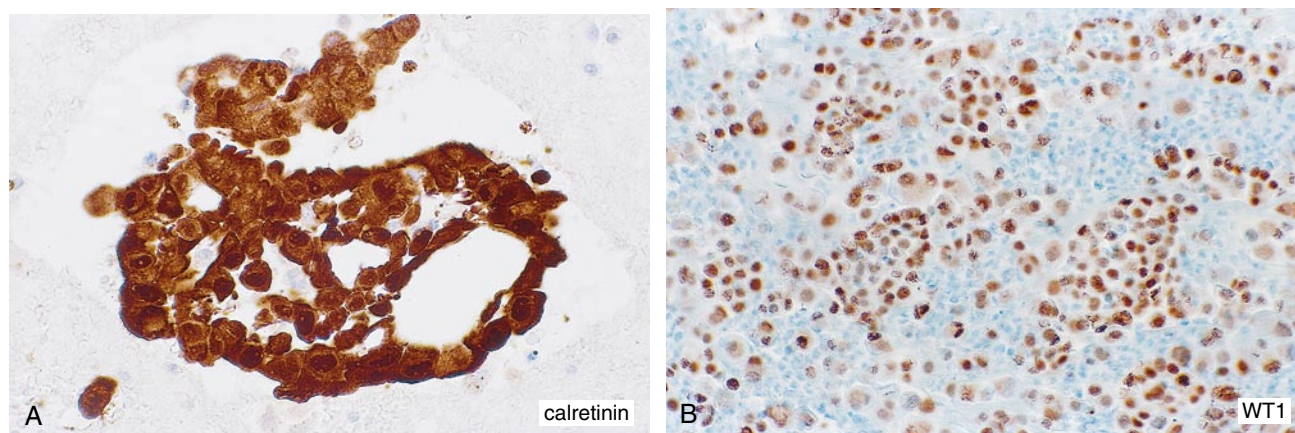


Figure 4.13 Immunoprofile of malignant mesothelioma. A, Mesotheliomas show nuclear and cytoplasmic staining for calretinin. B, There is usually nuclear immunoreactivity for Wilms tumor 1 protein (WT1).

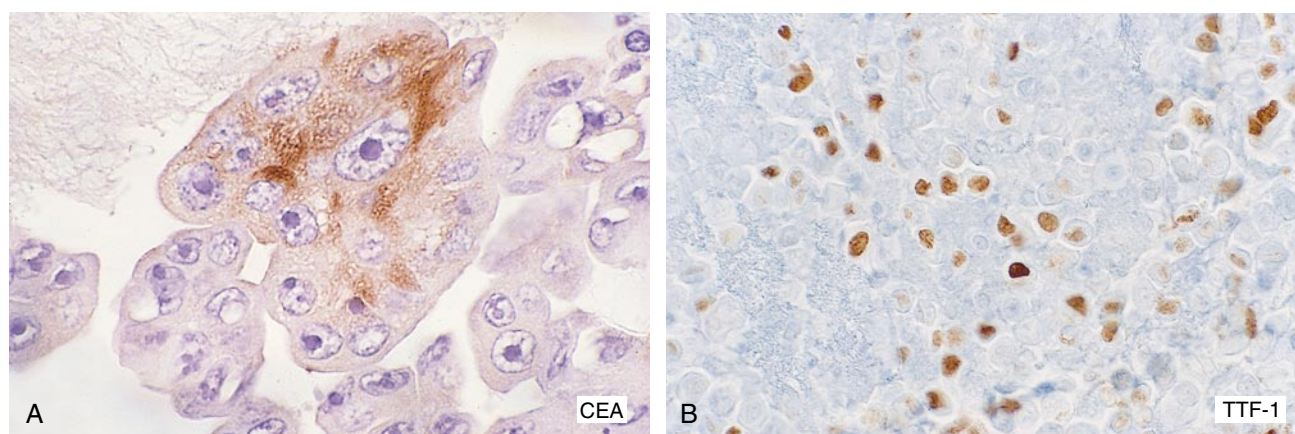


Figure 4.14 Immunoprofile of metastatic adenocarcinomas. A, Most adenocarcinomas are immunoreactive for one or more of the “carcinoma markers” like carcinoembryonic antigen ([CEA] shown here), but often only some of the cells are positive. B, Most adenocarcinomas of the lung are immunoreactive for thyroid transcription factor-1 (TTF-1), as are most thyroid cancers.

A positive staining reaction is nevertheless encountered in rare instances of mesothelioma.^{30,68} For this reason, it is wise to use a panel of histochemical and immunocytochemical stains from those listed in Table 4.2. A panel of four or five markers is sufficient in most cases and can be tailored to the particular differential diagnosis (Table 4.3).⁶⁶

Mesothelioma can also be distinguished from metastatic adenocarcinoma by electron microscopy; the microvilli of mesothelioma cells have a length to diameter ratio of 15:1 or greater, whereas those of adenocarcinoma have a smaller ratio.¹⁶ This method requires special fixation of the specimen for optimal results, however, and is rarely used.

Mesothelioma versus Squamous Cell Carcinoma. In effusions as in histologic sections, certain mesotheliomas can resemble a squamous cell carcinoma, particularly because both typically have dense cytoplasm. Immunohistochemistry can be helpful in this distinction (see Table 4.3)

TABLE 4.3 RECOMMENDED IMMUNOHISTOCHEMICAL PANELS BASED ON DIFFERENTIAL DIAGNOSIS*

Differential Diagnosis	Panel
Pleural mesothelioma versus lung adenocarcinoma	Mesothelial markers calretinin, WT1 and carcinoma markers MOC-31, Ber-EP4, B72.3, CEA, TTF-1
Peritoneal mesothelioma versus serous carcinoma	Mesothelial marker calretinin and carcinoma markers MOC-31 Ber-EP4, estrogen receptors
Mesothelioma versus squamous carcinoma	Mesothelioma markers calretinin, WT1 and carcinoma markers p63, MOC-31
Mesothelioma versus renal cell carcinoma	Mesothelioma markers calretinin, WT1 and carcinoma markers LeuM1 (CD15), renal cell carcinoma antigen

CEA, carcinoembryonic antigen; TTF-1, thyroid transcription factor-1; WT1, Wilms tumor protein 1.

*Modified from Ordonez NG: What are the current best immunohistochemical markers for the diagnosis of epithelioid mesothelioma? A review and update. *Hum Pathol* 2007;38(1):1-16.

Mesotheliomas versus Vascular Tumors. Primary vascular tumors of the lung (angiosarcomas and epithelioid hemangioendotheliomas) are rare but they can present with a pseudomesotheliomatous growth pattern that mimics mesotheliomas not just clinically and radiologically but also cytologically.⁶⁹ They arise in the lung but spread to involve the pleural surface in a diffuse pattern. Epithelioid hemangioendotheliomas bear an uncanny resemblance to epithelial mesotheliomas (Fig. 4.15). A panel of immunostains that includes vascular markers (CD31 and CD34) and mesothelial markers (calretinin and WT1) are helpful in this distinction.

Primary Effusion Lymphoma

Primary effusion (body-cavity based) lymphoma (PEL) is a rare subtype of diffuse large B-cell lymphoma that is associated with human herpes virus 8 (HHV-8) and presents as a pleural, pericardial, or peritoneal effusion.⁷⁰ Most cases have a null-cell immunophenotype; B-cell clonality is usually demonstrated by molecular studies. Rare PELs have a T-cell immunophenotype.^{71,72} All cases are positive for HHV-8; indeed, detection of HHV-8 is an essential step in confirming the diagnosis of PEL. By definition, this is a fluid-based malignancy without an associated mass lesion, lymphadenopathy, or organomegaly.⁷³ Most cases arise in the setting of human immunodeficiency virus (HIV) infection, but rare cases in transplant recipients have also been reported. The prognosis is poor: median survival is less than 6 months.^{73,74}

CYTOMORPHOLOGY OF PRIMARY EFFUSION LYMPHOMA:

- dispersed large cells
- round or irregular nucleus
- prominent nucleoli
- abundant basophilic cytoplasm (Romanowsky stain)

The cells are always large, ranging from plasmablastic or immunoblastic (round nucleus with prominent nucleolus) to anaplastic (large, irregular, multilobated nucleus) in morphology (Fig. 4.16A). Cytoplasm is abundant and may have a perinuclear halo or vacuoles. Mitoses and apoptotic bodies are present and can be numerous.⁷¹ The diagnosis of malignancy is straightforward. Given the usual clinical setting of an immunodeficiency, a lymphoma should be suspected; this can be confirmed by immunocytochemistry. PEL cells usually express CD45 (leukocyte common antigen) but are negative for the B-cell markers CD19 and CD20. Other lymphoid markers, like the activation marker CD30 and the plasma cell markers CD38 and CD138, are often present.⁷³ Demonstrating the presence of human HHV-8 is essential for diagnosis (see Fig. 4.16B).⁷² In most cases the neoplastic cells are co-infected with Epstein-Barr virus (EBV), best demonstrated by EBV-encoded RNA (EBER) in situ hybridization.⁷²

PEL cells are markedly atypical and obviously malignant. It is usually simply a question of classifying

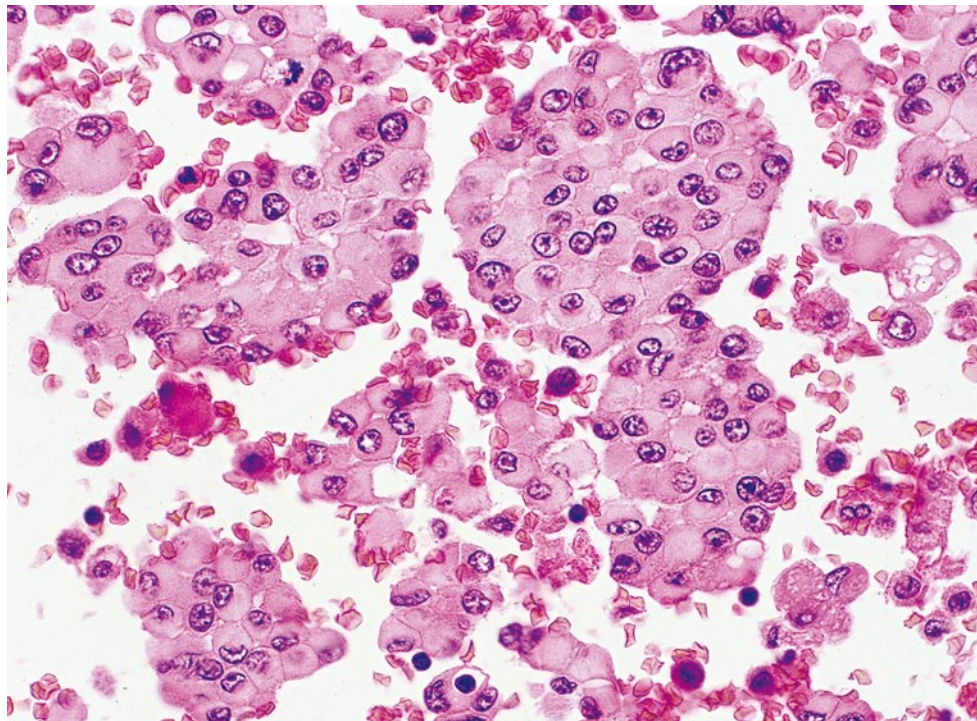


Figure 4.15 Epithelioid hemangioendothelioma of the lung (pleural fluid). This tumor is a good mimic of mesothelioma because the cells form large aggregates and have round, centrally placed nuclei and abundant cytoplasm.

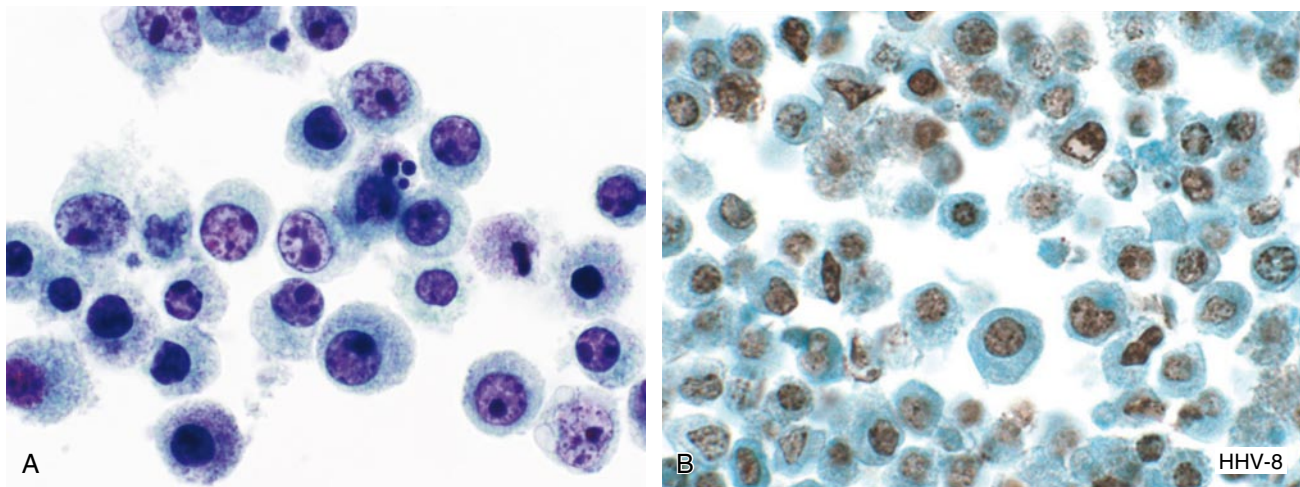


Figure 4.16 Primary effusion lymphoma. A, The malignant cells are large, with thick nuclear membranes, irregularly distributed chromatin, and prominent nucleoli. Apoptotic bodies are present. B, The presence of human herpes virus 8 (HHV-8), demonstrated here by immunohistochemistry, is a sine qua non of primary effusion lymphomas.

DIFFERENTIAL DIAGNOSIS OF PRIMARY EFFUSION LYMPHOMA:

- diffuse large B-cell lymphoma (not PEL)
- pyothorax-associated lymphoma
- anaplastic large cell lymphoma
- post-transplant lymphoproliferative disorder
- carcinoma
- melanoma

the malignancy. PEL is morphologically similar to the immunoblastic type of diffuse large B-cell lymphoma (DLBL). If the patient has a mass lesion, lymphadenopathy, or organomegaly along with the effusion, the lymphoma is not classified as a PEL but is instead a conventional DLBL. Immunohistochemistry can also distinguish these entities because DLBL expresses B-cell markers and is negative for HHV-8. *Pyothorax-associated lymphoma (PAL)* is a rare, EBV-associated subtype of DLBL that develops after longstanding chronic pleural inflammation, and there is usually an associated pleural mass. Like other DLBLs, it expresses B-cell markers and is negative for HHV-8. Anaplastic large cell lymphoma (ALCL) sometimes presents with a pleural effusion. The “hallmark cells” (with horse-shoe-shaped nuclei) typical of anaplastic large cell lymphoma (see Fig. 11.26) can be seen in some cases of PEL, but anaplastic large cell lymphoma is negative for HHV-8. In transplant recipients, the possibility of a post-transplant lymphoproliferative disorder (PTLD) should be considered (see Chapter 11). PTLDs are associated with EBV but are negative for HHV-8.⁷⁵ Carcinomas and melanoma can be excluded by the absence of immunoreactivity for keratins, S-100 protein, and HMB-45.

Metastatic Tumors

Adenocarcinoma

Metastatic carcinomas are by far the most common tumors found in effusions. Of these, adenocarcinomas are more common than squamous and small cell cancers. In most cases the carcinoma cells are easily recognized because, in one way or another, they are morphologically distinct from mesothelial cells (Fig. 4.17). This is the key to identifying them on cytologic preparations.

The cells of metastatic carcinoma are often but not necessarily larger, more pleomorphic, and more hyperchromatic than mesothelial cells. They can, in fact, be smaller and more uniform in size than the mesothelial cells around them. Most commonly, they are distinguished

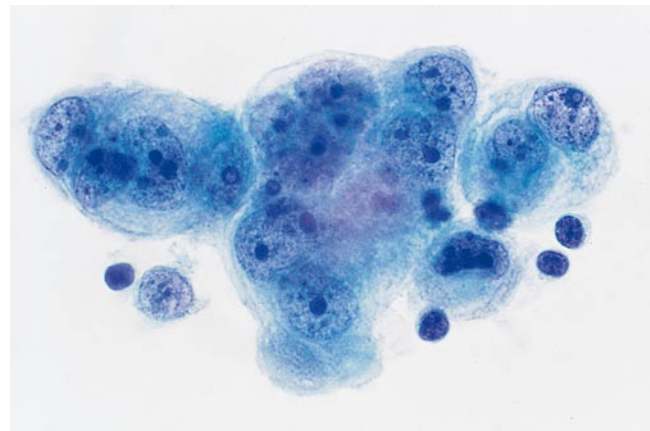


Figure 4.17 Adenocarcinoma of the lung (pleural fluid). Clusters of large, highly atypical cells like these are easily spotted and identified as malignant, but in the absence of a known primary, special stains might be needed for precise classification.

from benign mesothelial cells by their tendency to form large clusters (see Fig. 4.7). Other carcinomas, however, exfoliate in a noncohesive way, as isolated cells instead (Fig. 4.18). In such cases, cell size, increased nuclear-to-cytoplasmic ratio, irregular nuclear contours, prominent nucleoli, or coarsely textured chromatin distinguish them from mesothelial cells.

CYTOMORPHOLOGY OF ADENOCARCINOMAS:

- large spheres or single cells
- cytoplasmic vacuolization
- signet ring cells (gastric, breast)

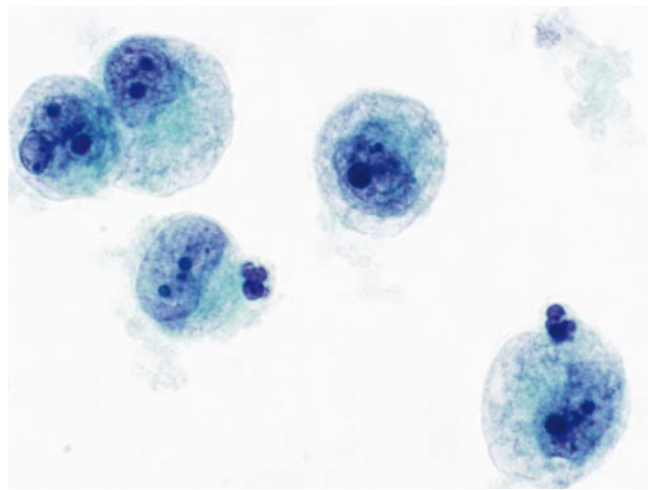


Figure 4.18 Adenocarcinoma of the lung (pleural fluid). In cases like this, the malignant cells can be more difficult to recognize because they are dispersed as isolated cells. Careful examination reveals markedly enlarged nucleoli and a cytoplasmic texture that is frothier and less dense than that of most mesothelial cells.

Adenocarcinomas exfoliate as large spheres or as isolated cells. Cytoplasmic vacuoles are often present, but they are not specific for adenocarcinomas; they are seen in some mesotheliomas and other tumors. Some morphologic clues can point to the site of origin of an adenocarcinoma. For example, abundant hollow spheres (“cannonballs”) are a common pattern in metastatic breast cancer (Fig. 4.19A and B). Malignant signet ring cells in an effusion point to an origin from stomach cancer, but other primary sites, such as the breast, are also possible (Fig. 4.20). Most colorectal cancers are composed of elongated cells with hyperchromatic nuclei arranged in acinar formations; individual cell necrosis is a common finding. Large cells with prominent nucleoli and abundant lacy, vacuolated cytoplasm are typical of clear cell carcinomas of the kidney and the female genital tract (Fig. 4.21). Most metastatic prostate cancers exfoliate as isolated cells or loosely cohesive groups of cells with prominent nucleoli. Some high-grade metastatic prostate cancers resemble small cell carcinoma.⁷⁶

Psammoma bodies are encountered in effusions caused by serous neoplasms of the ovary, fallopian tube, and endometrium; papillary carcinomas of the thyroid; adenocarcinomas of the lung; and mesotheliomas (Fig. 4.22). They are also seen in some benign proliferative reactions of the mesothelium, especially in the peritoneum,⁷⁷ but also the pericardium,²⁹ and by themselves should not be taken as evidence of malignancy.

When some mucinous adenocarcinomas, particularly those of the appendix, spread to involve the peritoneal surfaces, they produce a slow but relentless accumulation of extracellular mucin that eventually distends the peritoneal cavity, a condition known as *pseudomyxoma peritonei*. Peritoneal fluid from such patients is gelatinous and composed predominantly of mucin, which stains blue-green or purple with the Papanicolaou stain. Such specimens are often sparsely cellular and may contain only vacuolated histiocytes

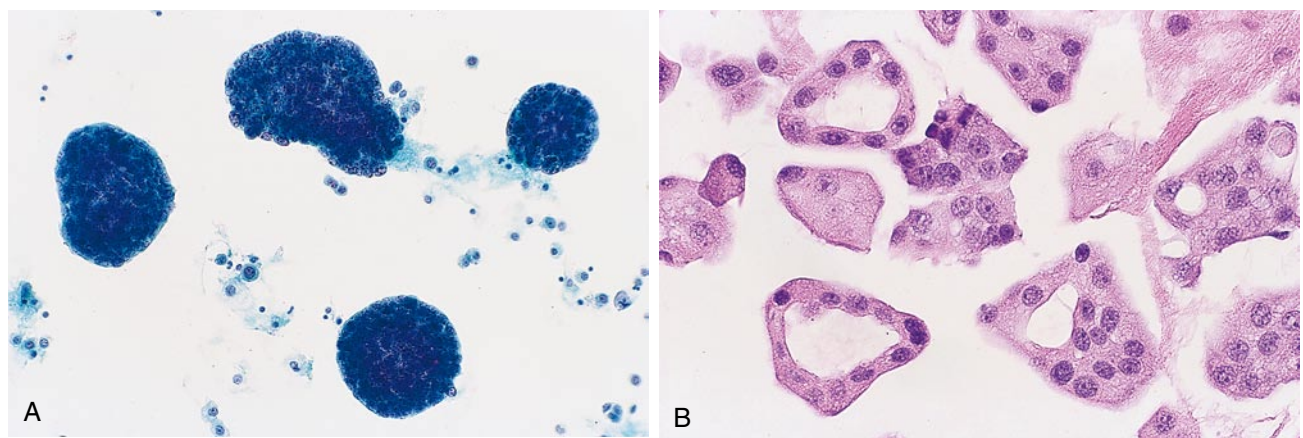


Figure 4.19 Ductal carcinoma of the breast (pleural fluid). Ductal cancers often exfoliate as large spheres of malignant cells (A, thinlayer slide), which may be hollow (B, cell block).

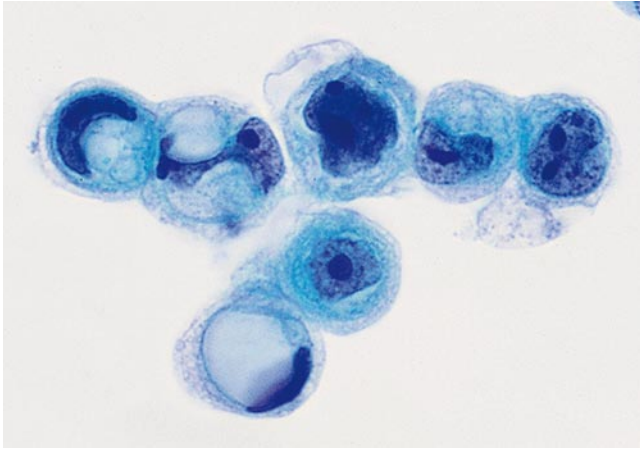


Figure 4.20 Adenocarcinoma of the stomach (pleural fluid). Large numbers of isolated signet ring cells are characteristic of many gastric cancers.

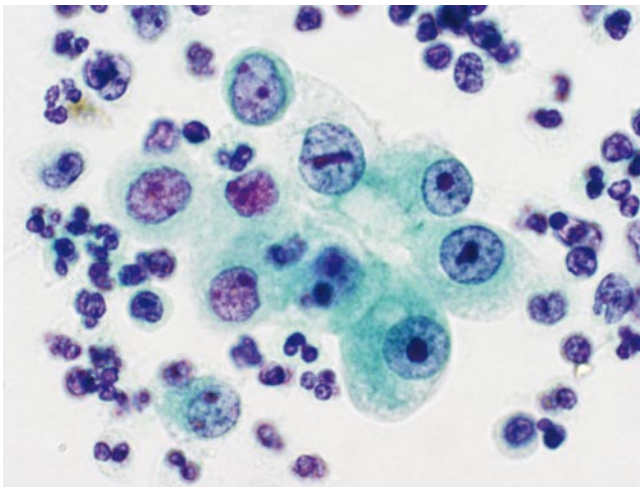


Figure 4.21 Clear cell carcinoma of the kidney (pleural fluid). Large cells with round nuclei, prominent nucleoli, and abundant granular and vacuolated cytoplasm are typical of renal cell carcinoma.

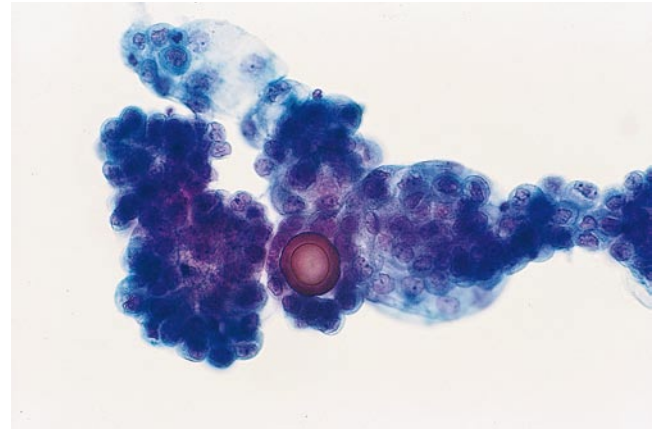


Figure 4.22 Metastatic papillary carcinoma of the thyroid (pleural fluid). This large irregular cluster of malignant cells is associated with a psammoma body.

called muciphages (Fig. 4.23A). The tumor cells in the peritoneum that produce the mucin (Fig. 4.23B) often comprise just a tiny fraction of the volume of the specimen and may not be present in the slides examined. When there are no definite malignant cells (just mucin and muciphages), only a presumptive diagnosis of malignancy can be offered.

DIFFERENTIAL DIAGNOSIS OF METASTATIC ADENOCARCINOMA:

- reactive mesothelial cells
- mesothelioma

It is usually easy to distinguish metastatic adenocarcinoma from reactive mesothelial cells. Malignant cells in cell block sections are frequently arranged in large clusters or are situated in lacunae (see Fig. 4.7). In some cases, however, metastatic adenocarcinoma cells are not easily recognized. A clear second population (morphologically distinct from mesothelial cells) may not

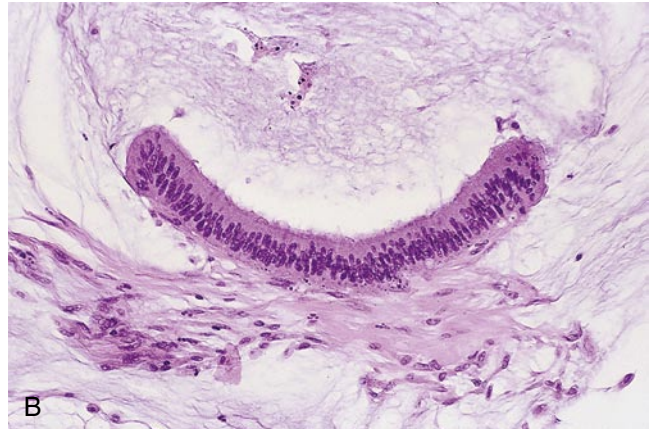
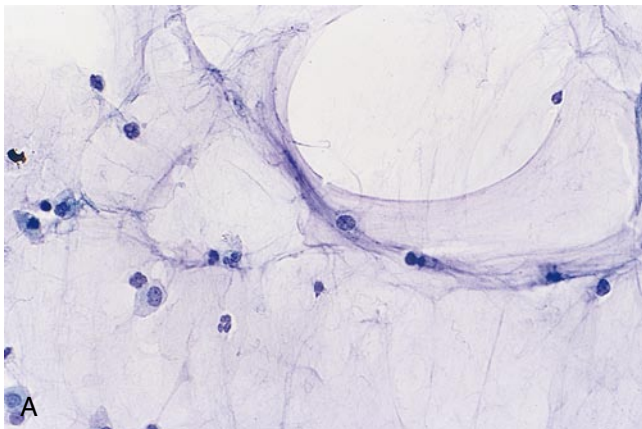


Figure 4.23 Pseudomyxoma peritonei (peritoneal fluid). A, Extracellular mucin is present in abundance, and stains blue or purple with the Papanicolaou stain. Malignant cells are often not seen; the few cells in this field are histiocytes. B, In this case, rare strips of neoplastic epithelium were identified in cell block sections.

be apparent; the tumor cells might mimic mesothelial cells, or the suspicious cells might be few in number or poorly preserved. In any doubtful case, special stains for mucin and immunohistochemistry for carcinoma markers (see Table 4.2) are helpful for distinguishing reactive mesothelial cells (negative) from adenocarcinomas (positive).^{28,65} A panel of four or five immunomarkers is sufficient in most cases. The panel can be selected based on clinical history (Table 4.3 can be applied for this purpose) and might include, in addition to carcinoma markers, two mesothelial markers like calretinin and WT-1.⁶⁶

Lobular carcinoma of the breast is perhaps the most subtle of all the adenocarcinomas; with only routine stains, the cells of this tumor can be impossible to distinguish from histiocytes and mesothelial cells (Fig. 4.24A). For this reason, it is advisable to do special stains for mucin (mucicarmine and PAS-D) and a limited panel of immunostains (e.g., CEA, gross cystic disease fluid protein, estrogen and progesterone receptors) on any effusion from a patient with lobular breast cancer (Fig. 4.24B).

Immunohistochemical markers are also helpful in patients with a tumor of unknown primary or a history of two or more neoplasms. Useful tissue-specific antigens include TTF-1 (for adenocarcinoma and small cell carcinoma of the lung and thyroid cancers, see Fig. 4.14B),⁶⁷ estrogen and progesterone receptors (for breast and gynecologic malignancies), thyroglobulin, prostate-specific antigen (PSA), and prostatic acid phosphatase (PAP). Negative staining for both prostate-specific antigen and prostatic acid phosphatase does not exclude metastatic prostatic cancer, however, because less than 50% of metastatic prostate cancers in pleural effusions are immunoreactive for these markers.⁷⁶

The differential diagnosis of adenocarcinoma and mesothelioma has been discussed at length previously. Immunostains are indispensable for a definitive diagnosis (see Table 4.3)

Squamous Cell Carcinoma

Squamous cell carcinomas (SQCs) rarely metastasize to the pleura, pericardium, or peritoneum. Those that do are most commonly carcinomas of the lung, larynx, and female genital tract.⁷⁸ The primary tumor is known in virtually all cases before an effusion develops.

CYTOMORPHOLOGY OF SQUAMOUS CELL CARCINOMA:

- large clusters or isolated cells
- keratinized or nonkeratinized
- dense cytoplasm

The cells of squamous cell carcinoma are arranged either in large clusters (Figs 4.25A and B, 4.26) or as isolated cells. The cytologic appearance differs depending on the degree of keratinization of the tumor. The cytoplasm is usually dense and occasionally orangeophilic. Cells with a tadpole or spindle shape are uncommon. Nuclei are enlarged, hyperchromatic, and coarsely granular; nucleoli are usually not prominent. Nuclear pyknosis and karyorrhexis are seen in some cases. Anucleated squames may be present. Cytoplasmic vacuolization is sometimes seen, and should not be interpreted as indicative of glandular differentiation.⁷⁸ A panel of immunostains that includes p63, MOC-31, and the mesothelial markers calretinin and WT-1 can be useful to distinguish squamous cell carcinoma from reactive mesothelial cells and mesothelioma (see Table 4.3).

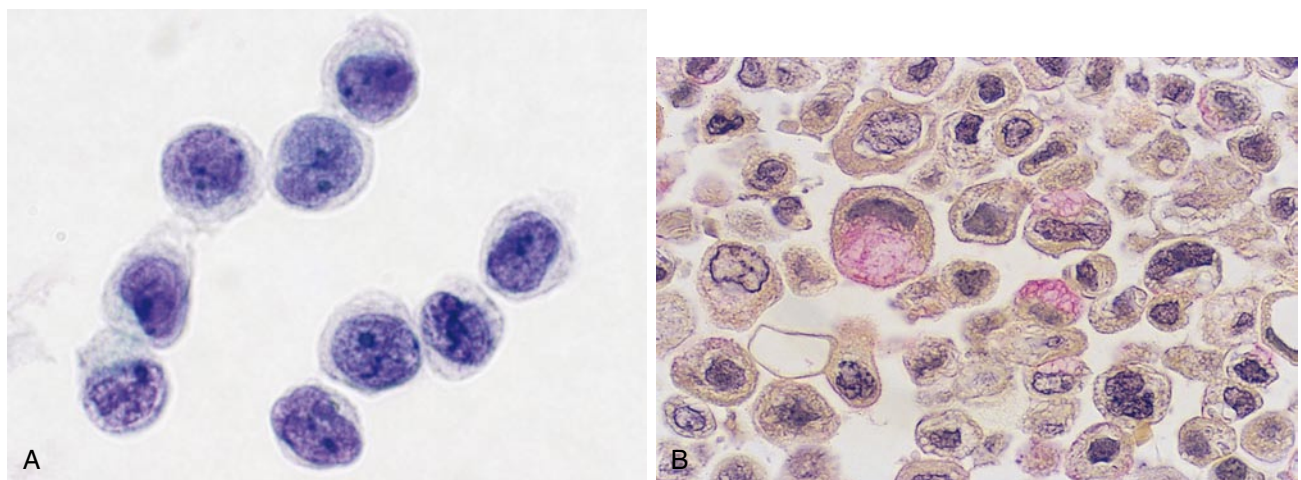


Figure 4.24 Lobular carcinoma of the breast (pleural fluid). A, These malignant cells are extremely difficult to recognize because they resemble histiocytes or mesothelial cells (Papanicolaou stain). B, A mucicarmine stain reveals focal intracytoplasmic mucin.

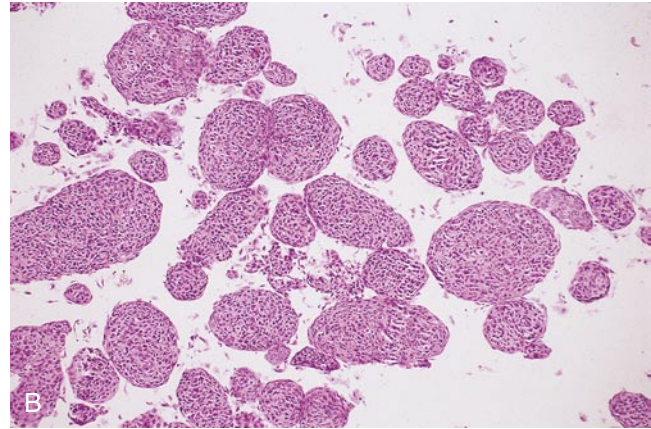
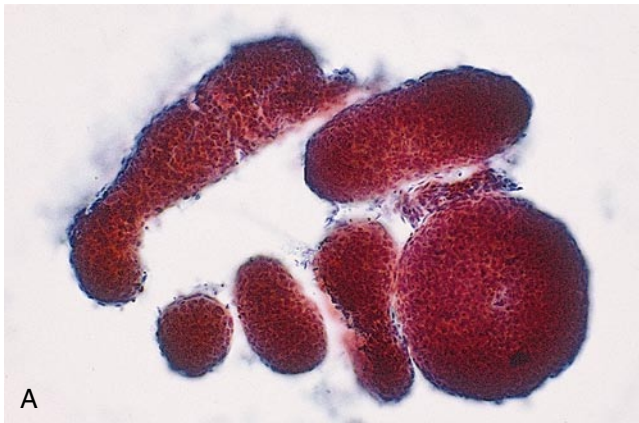


Figure 4.25 Squamous cell carcinoma of the cervix (pericardial fluid). Nonkeratinizing squamous cell cancers shed large spheres of malignant cells. A, Cytocentrifuge preparation. B, Cell block preparation.

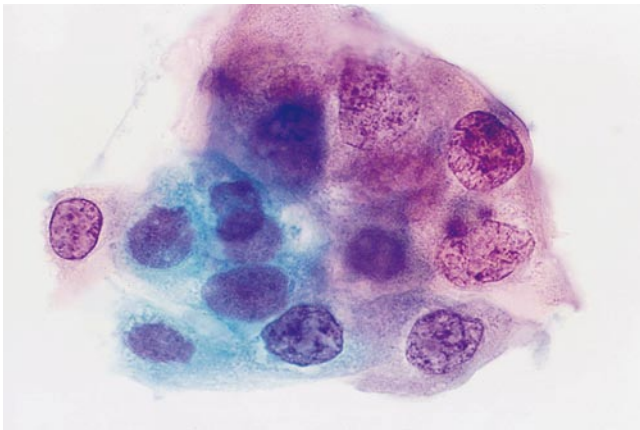


Figure 4.26 Squamous cell carcinoma of the lung (pleural fluid). The malignant cells have coarsely textured chromatin and plate-like cytoplasm.

Small Cell Carcinoma

Small cell carcinoma of the lung, despite its predilection for widespread metastases, causes a pleural effusion in less than 3% of patients.⁷⁹

CYTOMORPHOLOGY OF SMALL CELL CARCINOMA:

- small cells (isolated, in chains and clusters)
- nuclear molding
- scant cytoplasm

The cells are dispersed as isolated cells and arranged in clusters and chains. They are small, with a diameter approximately two to three times that of small lymphocytes. Cytoplasm is scant. The nuclei are dark and have a finely granular chromatin texture; nucleoli are inconspicuous. Karyorrhexis is common. When arranged in groups, the cells are crescent shaped and angulated as they wrap themselves around one another (Fig. 4.27).

The differential diagnosis includes lymphoma. The cells of small cell carcinoma are distinguished by their tendency to form clusters. Although the nuclei of lymphoma cells can be irregular in shape, they are rarely sharply angular like the cells of small cell carcinoma. Most small cell lung cancers, unlike lymphomas, are immunoreactive for TTF-1, but a negative result does not exclude the diagnosis. The differential diagnosis also includes other small cell malignancies like small cell carcinoma of the prostate⁷⁶ and Merkel cell carcinoma. In most cases, the clinical history of a specific malignancy points in the correct direction. Immunohistochemistry can be helpful if needed: For example, small cell carcinomas are immunoreactive for CK7 and negative for CK20; the reverse is true for Merkel cell carcinoma.

Melanoma

Most malignant melanomas arise in the skin, but extra-cutaneous tumors, such as ocular melanomas, do occur. In 5% of cases, patients present with metastatic disease

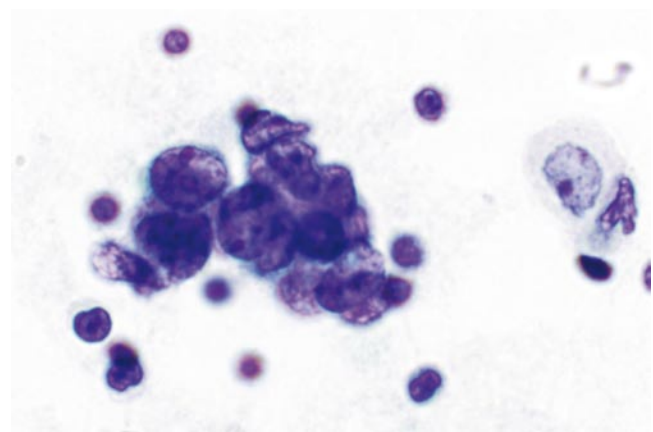


Figure 4.27 Small cell carcinoma of the lung (pleural fluid). Some of the small malignant cells are dispersed as isolated cells, whereas others are molded together in clusters. The mesothelial cell on the right provides a size comparison.

without a known primary or with only a remote history of a pigmented cutaneous lesion. To compound the problem, the diagnosis of melanoma can be subtle in effusions. The malignant cells resemble mesothelial cells: they are often dispersed as isolated round cells with prominent nucleoli (Fig. 4.28A). In some cases, cells show a fine brown cytoplasmic pigmentation or intranuclear pseudoinclusions. Cell clusters are uncommon (Fig. 4.28B), and cell blocks rarely show pericellular lacunae. The distinction from reactive mesothelial cells is difficult in cases that show little nuclear pleomorphism and hyperchromasia and no cytoplasmic pigmentation.⁸⁰ Immunocytochemistry is helpful; melanomas are reactive for S-100 protein and HMB-45, and negative for keratin proteins.

Non-Hodgkin Lymphoma

A malignant effusion complicates the course of disease in many patients with non-Hodgkin lymphoma. In fact, 10% to 15% of malignant effusions are caused by lymphoma; the proportion is significantly higher in the pediatric population.^{23,81} Most represent secondary involvement of serosal surfaces; few are PELs. Certain subtypes have a greater propensity for involving serosal surfaces (e.g., lymphoblastic lymphoma).

Cytologic preparations are highly cellular and composed of dispersed lymphoid cells. Lacunar spaces are not seen in cell block sections with lymphomas. In many cases, mesothelial cells are conspicuously absent. The cells of DLBLs are easiest to recognize because they are larger than histiocytes and their nucleoli are usually prominent (Fig. 4.29). Nuclei are round or highly irregular, and the chromatin is coarsely textured. Cytoplasm is often abundant, pale, and vacuolated. The cells of small cell lymphomas are only slightly larger than normal lymphocytes. Those of follicular lymphomas have irregular

and cleaved nuclei and scant cytoplasm. Lymphoblastic lymphomas are also composed of relatively small cells with round or irregularly shaped nuclei, finely dispersed chromatin, and scant or moderate amounts of cytoplasm (Fig. 4.30). Small lymphocytic lymphoma rarely involves serosal cavities. Burkitt and Burkitt-like lymphomas are high-grade neoplasms composed of lymphoid cells of intermediate size with round nuclei, prominent nucleoli, and coarsely textured chromatin. Mitoses are numerous.

Karyorrhexis is a conspicuous feature of many lymphomas (see Fig. 4.29). It is seen in both treated and untreated patients and is an uncommon finding in benign effusions or malignant effusions resulting from other tumors.

Precise classification of the lymphoma based on its morphology is rarely necessary with effusions because most patients have biopsy-proven disease before the effusion develops. Nevertheless, some degree of subclassification is usually possible based on the size of the cells, the degree of nuclear membrane irregularity (cleaved or noncleaved), and whether the cells resemble lymphoblasts or Burkitt cells. Such features can be appreciated especially well on air-dried Romanowsky-stained preparations (Fig. 4.30).

DIFFERENTIAL DIAGNOSIS OF SECONDARY INVOLVEMENT BY LYMPHOMA:

- PEL
- benign lymphocytic effusion
- PTLD
- carcinoma
- mesothelioma
- melanoma
- small round blue-cell tumors

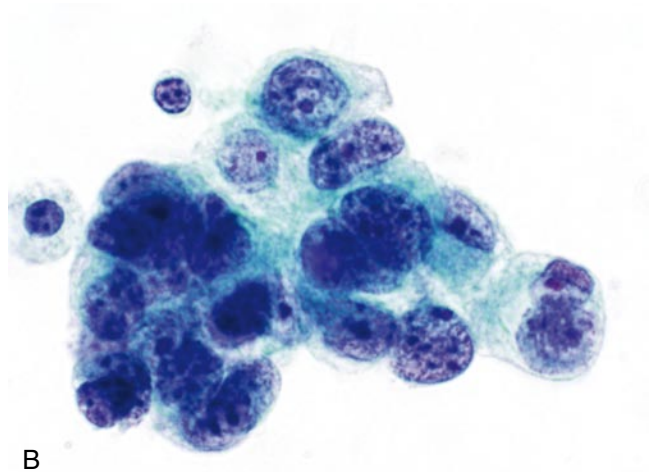
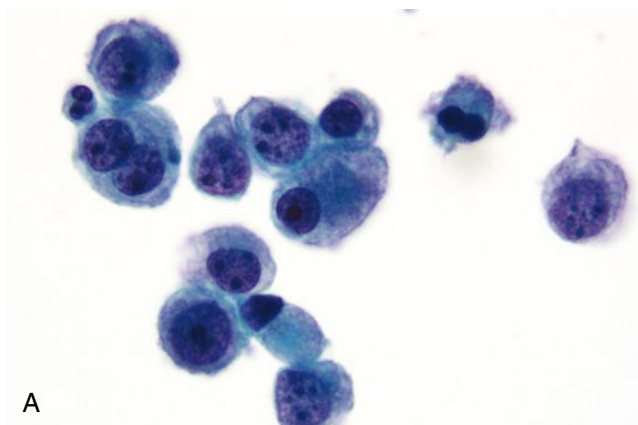


Figure 4.28 Melanoma (pleural fluid). A, Nonpigmented melanoma cells are usually isolated, not clustered, and resemble mesothelial cells. B, Less often, they aggregate in large clusters.

Figure 4.29 Non Hodgkin lymphoma: diffuse large B-cell type (peritoneal fluid). The lymphoma cells have predominantly round nuclei with prominent nucleoli. Note the karyopyknosis and karyorrhexis, characteristic of most lymphomas.

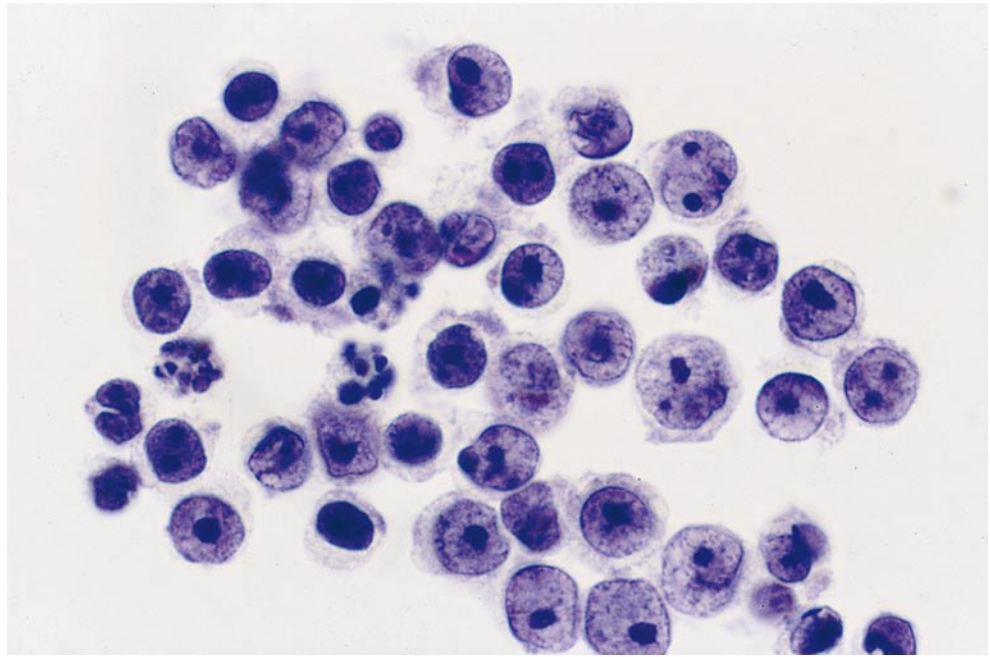
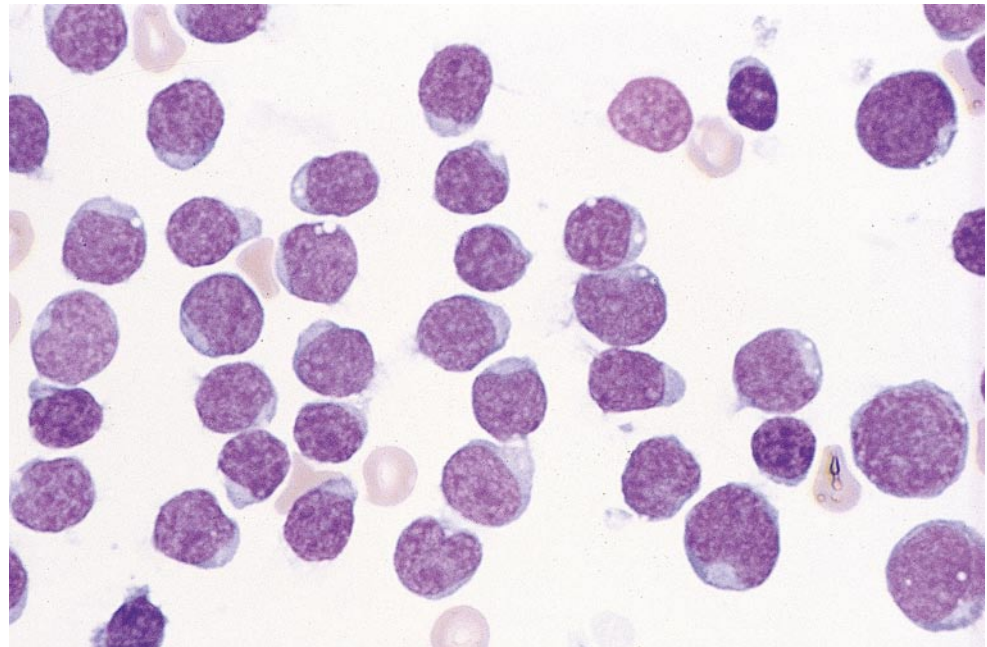


Figure 4.30 Lymphoblastic lymphoma. Because lymphoblastic lymphoma cells are only slightly larger than normal lymphocytes, they are easily misdiagnosed. They are recognized by their more finely dispersed chromatin texture, better appreciated on air-dried preparations (Wright-Giemsa stain).



Secondary involvement by a large-cell non-Hodgkin lymphoma like DLBL or anaplastic large cell lymphoma can be morphologically indistinguishable from a PEL. The absence of a documented primary or of any mass lesion, lymphadenopathy, or organomegaly in a patient who is immunocompromised should raise the possibility of PEL, which can be confirmed by demonstrating the presence of HHV-8. The differential diagnosis of secondary involvement by a lymphoma composed predominantly of small cells (e.g., lymphoblastic lymphoma)

phoma) includes a benign lymphocytic effusion like that resulting from tuberculosis. The diagnosis of tuberculosis should be considered if there are suspicious clinical findings and the fluid is composed predominantly of small mature lymphocytes. The cells of small lymphocytic lymphoma are virtually impossible to distinguish from small, mature lymphocytes with Papanicolaou-stained preparations, even with the help of computer-assisted morphometry.⁸² Given that serosal involvement by small lymphocytic lymphoma (or its

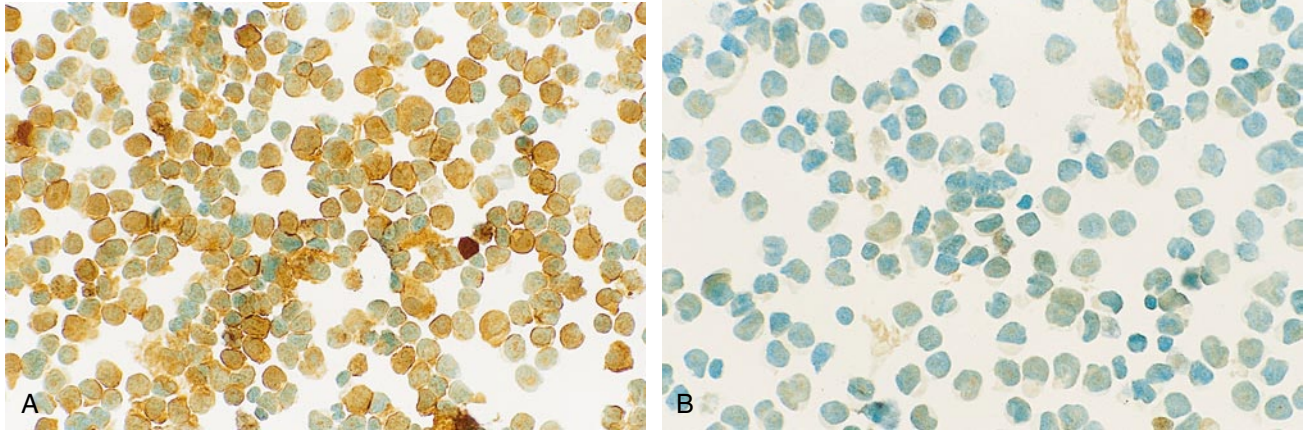


Figure 4.31 Non-Hodgkin lymphoma. In doubtful cases, immunostains for immunoglobulin light chains, performed on air-dried cytocentrifuge preparations, can be helpful. Here the malignant lymphoid cells are immunoreactive for κ light chains A, and negative for λ light chains B.

leukemic counterpart, chronic lymphocytic leukemia) is a highly rare event (some authors who have reviewed large series of malignant lymphomas could find no autopsy-documented cases),⁸³ it is wise to document immunoglobulin light chain restriction before rendering a diagnosis of malignancy. κ and λ light chain expression can be examined on air-dried cytocentrifuge preparations using immunocytochemistry⁷ or by flow cytometry.¹³ Clonal excess is determined in many cases by a simple inspection of the proportion of B cells that express κ or λ light chains (Fig. 4.31A and B). This method is a useful adjunct for the cytologic evaluation of lymphocyte-rich effusions that are cytologically equivocal for malignancy.¹⁴ In the case of the less common T-cell lymphoma, a T-cell phenotype with aberrant markers can be demonstrated by immunocytochemistry or flow cytometry, confirming a malignant diagnosis.⁷

In a transplant recipient, the possibility of a PTLD must be considered whenever an effusion contains a prominent lymphoid population. PTLDs are associated with EBV, best demonstrated by EBV-encoded RNA in situ hybridization and are negative for HHV-8.

Lymphomas are rarely confused with other malignancies because other tumors tend to form cell clusters in effusions. In some preparations, a crowding of the lymphoma cells mimics cluster formation, but such artifactual clustering is usually recognizable for what it is. Conversely, some carcinomas, mesotheliomas, and melanomas shed in a noncohesive pattern and can resemble the cells of a large-cell lymphoma (see Figs. 4.8C, 4.18). If there is any doubt concerning the lymphoid nature of a malignant effusion, a panel of immunomarkers (e.g., leukocyte common antigen, keratin proteins, calretinin, WT1, S-100, HMB-45) can be helpful. Similarly, small round blue cell tumors (e.g., neuroblastoma, Ewing sarcoma/PNET) can be ruled out with the appropriate immunohistochemical panel.

Hodgkin Lymphoma

Patients with Hodgkin lymphoma can develop benign and malignant effusions. The more common benign effusions are likely a result of thoracic duct obstruction or impaired lymphatic drainage resulting from a tumor that is nearby but not directly involving the serosal surface. Malignant effusions are relatively uncommon and are almost never the initial manifestation of the disease.

The cytologic hallmark is the Reed-Sternberg cell, a large, multinucleated cell with huge inclusion-like nucleoli (Fig. 4.32). Mononuclear variants are often present, together with a mixed population of inflammatory cells that includes lymphocytes, plasma cells, eosinophils, neutrophils, and histiocytes.

In a patient with a history of Hodgkin lymphoma, a fluid composed of a mixed population of inflammatory cells but without Reed-Sternberg cells is considered suggestive of malignancy.

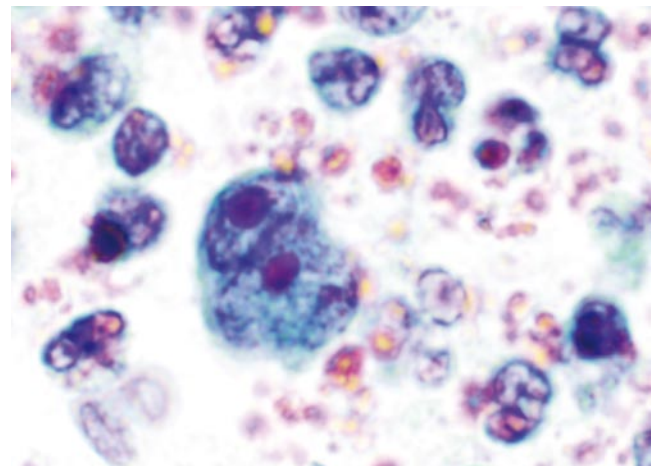


Figure 4.32 Hodgkin lymphoma (pleural fluid). The classic Reed-Sternberg cell is a large, multinucleated cell, with prominent nucleoli.

Multiple Myeloma

Multiple myeloma rarely involves the serosal cavities. The pleura is involved more commonly than the peritoneum or pericardium. Pleural involvement is sometimes a direct extension of the tumor from an erosive rib lesion.

The degree of plasmacytoid differentiation varies from one tumor to another. The cells are large and dispersed as isolated cells. Nuclei are round and have coarsely textured chromatin with prominent nucleoli. Cytoplasm is usually abundant, nuclei are eccentrically positioned within the cell, and there is a perinuclear clear zone (Fig. 4.33A). In poorly differentiated tumors the cells have less cytoplasm and vary in size and shape.⁸⁴

Immunocytochemistry reveals monotypic expression of either κ or λ light chains. Cell block sections are particularly well suited because the immunoglobulin is cytoplasmic in these tumors. Curiously, although the cells are negative for keratin proteins, they often express epithelial membrane antigen.⁸⁴ They are also positive for the plasma cell marker CD138 (Fig. 4.33B).

Acute and Chronic Leukemias

Acute lymphoblastic and myeloblastic leukemias occasionally involve the serosal surfaces. Blasts are round, two or three times the diameter of lymphocytes, and dispersed as isolated cells. The nuclei are round or irregularly shaped. The chromatin is pale and finely dispersed, and nucleoli are usually prominent.

Lymphoblasts cannot be distinguished from myeloblasts on Papanicolaou-stained preparations, but this is rarely if ever important because the type of leukemia has usually been determined long before the patient develops an effusion. Auer rods, pink rodlike structures in the cytoplasm, are diagnostic of myeloid differentiation, and can be appreciated on air-dried Romanowsky-stained preparations.

The cells of chronic lymphocytic leukemia are indistinguishable from small, mature lymphocytes. Thus, immunophenotyping is necessary to establish this diagnosis.

In patients with circulating blasts, the possibility that the fluid was contaminated by peripheral blood during a traumatic tap is likely if the specimen contains red blood cells.

Sarcomas

Virtually any sarcoma can metastasize to the serosal surfaces, although they do so much less frequently than other tumors. When they do, it is usually late in the course of the disease. Those that have been reported in effusions range in morphology from spindle cell sarcomas, like leiomyosarcoma, synovial sarcoma, and malignant nerve sheath tumors, to small round cell sarcomas, like Ewing sarcoma/PNET, neuroblastoma, and embryonal rhabdomyosarcoma.⁴⁶

The cytomorphology of sarcomas in effusions is highly variable, depending on the sarcoma involved. The cells may be cohesive, exfoliating as tissue fragments (see Fig. 4.15), or they may be dispersed as isolated cells (Fig. 4.34), and the individual cells can be small or large. Small round cell sarcomas, like embryonal rhabdomyosarcoma, exfoliate predominantly as dispersed cells, mimicking the pattern of a non-Hodgkin lymphoma. Immunocytochemistry is helpful in resolving equivocal cases.

Germ Cell Tumors

Like sarcomas, germ cell tumors rarely cause malignant effusions. When they do, it is usually late in the course of widely disseminated disease.

The *seminoma* of the testis, and its counterpart, the *dysgerminoma* of the ovary, are composed of dyshesive uniform cells that resemble enlarged mesothelial cells. They have less cytoplasm than mesothelial cells, however, and more prominent nucleoli.

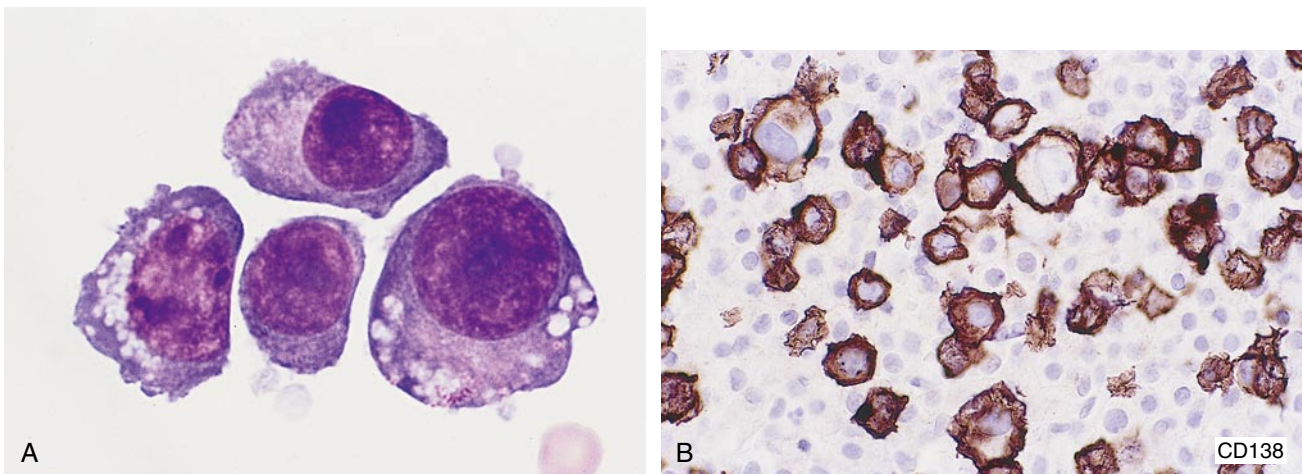


Figure 4.33 Multiple myeloma (pleural fluid). A, The fluid is composed almost exclusively of malignant plasma cells with abundant cytoplasm and eccentrically placed nuclei. B, The malignant cells are immunoreactive for CD138.

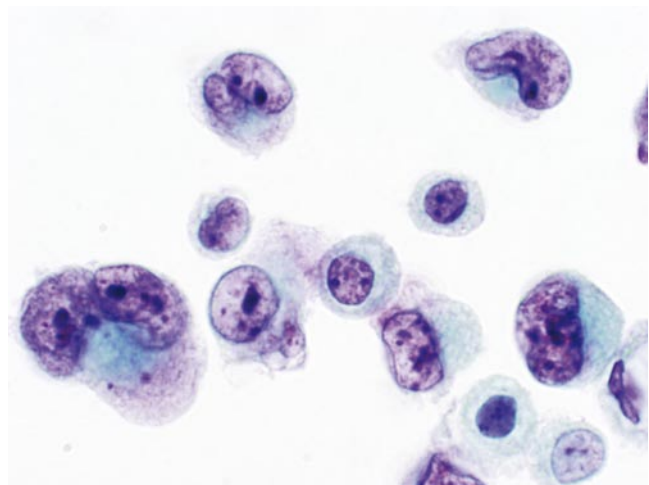


Figure 4.34 Pleomorphic rhabdomyosarcoma. The malignant cells are isolated. Binucleation, eccentrically placed nuclei, and dense cytoplasm are typical of many rhabdomyosarcomas.

Nonseminomatous germ cell tumors, which include embryonal carcinoma, endodermal sinus (yolk sac) tumor, choriocarcinoma, and malignant teratoma, are composed of large pleomorphic cells with pale, finely granular chromatin and prominent, often multiple, nucleoli. The cells are morphologically indistinguishable from poorly differentiated large cell carcinomas. Immunocytochemistry for placental alkaline phosphatase is helpful in distinguishing nonseminomatous germ cell tumors (positive) from large cell carcinomas (negative). Special studies, however, are rarely necessary because these tumors rarely manifest themselves initially as a malignant effusion.

Rarely, *sex cord-stromal tumors* can also involve serosal surfaces and cause an effusion, sometimes many years after treatment of the primary tumor (Fig. 4.35). Immunohistochemistry for inhibin, a marker of many sex cord-stromal tumors, along with comparison of the effusion specimen with sections from the original tumor, can be indispensable for correct interpretation.

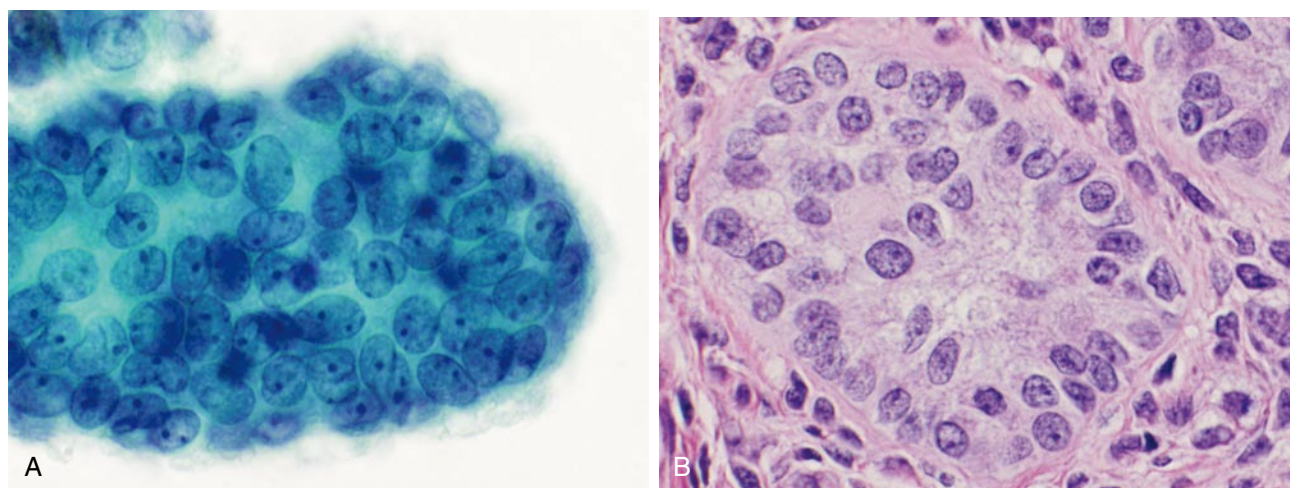


Figure 4.35 Adult granulosa cell tumor (ascites). A, The malignant cells are uniform and bland and resemble benign mesothelial cells. The arrangement in large clusters was suspicious, as was the prominence of nuclear grooves and focal follicle formation. B, Comparison with the original histologic sections was helpful in establishing the diagnosis.

REFERENCES

1. Thomsen TW, DeLaPena J, Setnik GS: Videos in clinical medicine. Thoracentesis. *N Engl J Med* 2006;355(15):16e.
2. Covey AM: Management of malignant pleural effusions and ascites. *J Support Oncol* 2005;3(2):169-173 76.
3. Roffe BD, Wagner FH, Derewicz HJ, Gill GW: Heparinized bottles for the collection of body cavity fluids in cytopathology. *Am J Hosp Pharm* 1979;36:211-214.
4. Manosca F, Schinstine M, Fetsch PA, et al.: Diagnostic effects of prolonged storage on fresh effusion samples. *Diagn Cytopathol* 2007;35(1):6-11.
5. Ylagan LR, Zhai J: The value of ThinPrep and cytospin preparation in pleural effusion cytological diagnosis of mesothelioma and adenocarcinoma. *Diagn Cytopathol* 2005;32(3):137-144.
6. Gabriel C, Achten R, Drijkoningen M: Use of liquid-based cytology in serous fluids: A comparison with conventional cytopreparatory techniques. *Acta Cytol* 2004;48(6):825-835.
7. Martin SE, Zhang HZ, Magyarosy E, et al.: Immunologic methods in cytology: Definitive diagnosis of non-Hodgkin's lymphomas using immunologic markers for T- and B-cells. *Am J Clin Pathol* 1984;82:666-673.
8. De Girolami E: Applications of plasma-thrombin cell block in diagnostic cytology. II: Digestive and respiratory tracts, breast and effusions. *Pathol Annu* 1977;12(Part 2):91-110.
9. De Girolami E: Applications of plasma-thrombin cell block in diagnostic cytology. I: Female genital and urinary tracts. *Pathol Annu* 1977;12(Part 1):251-275.

10. Naylor B: Pleural, peritoneal, and pericardial fluids. In Bibbo M (ed): Comprehensive Cytopathology, 2nd ed. Philadelphia, WB Saunders, 1997, pp. 551-621.
11. Starr RL, Sherman ME: The value of multiple preparations in the diagnosis of malignant pleural effusions: A cost-benefit analysis. *Acta Cytol* 1991;35:533-537.
12. Fetsch PA, Simsir A, Brosky K, Abati A: Comparison of three commonly used cytologic preparations in effusion immunocytochemistry. *Diagn Cytopathol* 2002;26(1):61-66.
13. Czader M, Ali SZ: Flow cytometry as an adjunct to cytomorphologic analysis of serous effusions. *Diagn Cytopathol* 2003;29(2):74-78.
14. Ibrahim RE, Teich D, Smith BR, et al.: Flow cytometric surface light chain analysis of lymphocyte rich effusions: A useful adjunct to cytologic diagnosis. *Cancer* 1989;63:2024-2029.
15. Zarbo RJ: Flow cytometric DNA analysis of effusions: a new test seeking validation. *Am J Clin Pathol* 1991;95:2-4.
16. Kobzik L, Antman KH, Warhol MJ: The distinction of mesothelioma from adenocarcinoma in malignant effusions by electron microscopy. *Acta Cytol* 1985;29:219-225.
17. Granados R, Cibas ES, Fletcher JA: Cytogenetic analysis of effusions for malignant mesothelioma: A diagnostic adjunct to cytology. *Acta Cytol* 1994;38:711-717.
18. Factor RE, Dal Cin P, Fletcher JA, Cibas ES: The value of cytogenetics and molecular cytogenetics as adjuncts to cytology in the diagnosis of malignant mesothelioma in pleural and peritoneal fluids. *Mod Pathol* 2008;21:72A.
19. Motherby H, Nadjari B, Friegel P, et al.: Diagnostic accuracy of effusion cytology. *Diagn Cytopathol* 1999;20:350-357.
20. Spriggs AI, Boddington MM: Atlas of serous fluid cytopathology: A guide to cells of pleural, pericardial, peritoneal and hydrocele fluids. In Gresham GA (ed): Current Histopathology Series. Dordrecht, The Netherlands, Kluwer Academic Publishers, 1989, p. 14.
21. Nance KV, Shermer RW, Askin FB: Diagnostic efficacy of pleural biopsy as compared with that of pleural fluid examination. *Mod Pathol* 1991;4:320-324.
22. Garcia LW, Ducatman BS, Wang HH: The value of multiple fluid specimens in the cytological diagnosis of malignancy. *Mod Pathol* 1994;7:665-668.
23. Johnston WW: The malignant pleural effusion: A review of cytopathologic diagnoses of 584 specimens from 472 consecutive patients. *Cancer* 1985;56:905-909.
24. Light RW: Clinical practice. Pleural effusion. *N Engl J Med* 2002;346(25):1971-1977.
25. Kutty CPK, Remeniuk E, Varkey B: Malignant-appearing cells in pleural effusion due to pancreatitis: Case report and literature review. *Acta Cytol* 1981;25:412-416.
26. Hsu C: Cytologic detection of malignancy in pleural effusion: A review of 5255 samples from 3811 patients. *Diagn Cytopathol* 1987;3:8-13.
27. Hallman JR, Geisinger KR: Cytology of fluids from pleural, peritoneal and pericardial cavities in children: A comprehensive survey. *Acta Cytol* 1994;38:209-217.
28. Esteban JM, Yokota S, Husain S, Battifora H: Immunocytochemical profile of benign and carcinomatous effusions. *Am J Clin Pathol* 1990;94:698-705.
29. Zakowski M, Ianuela-Shanerman A: Cytology of pericardial effusions in AIDS patients. *Diagn Cytopathol* 1993;9:266-269.
30. Churg A, Cagle PT, Roggli VL (eds): Tumors of the Serosal Membranes. Silver Spring, Md, ARP Press, 2006.
31. Choi YL, Song SY: Cytologic clue of so-called nodular histiocytic hyperplasia of the pleura. *Diagn Cytopathol* 2001;24(4):256-259.
32. Drury M, Anderson W, Heffner JE: Diagnostic value of pleural fluid cytology in occult Boerhaave's syndrome. *Chest* 1992;102:976-978.
33. Kalomenidis I, Light RW: Pathogenesis of the eosinophilic pleural effusions. *Curr Opin Pulm Med* 2004;10(4):289-293.
34. Romero Candeira S, Hernandez Blasco L, Soler MJ, et al.: Biochemical and cytologic characteristics of pleural effusions secondary to pulmonary embolism. *Chest* 2002;121(2):465-469.
35. Veress JF, Koss LG, Schreiber K: Eosinophilic pleural effusions. *Acta Cytol* 1979;32:40-44.
36. Naylor B, Novak PM: Charcot-Leyden crystals in pleural fluids. *Acta Cytol* 1985;29:781-784.
37. Spieler P: The cytologic diagnosis of tuberculosis in pleural effusions. *Acta Cytol* 1979;23:374-378.
38. Renshaw AA, Dean BR, Antman KH, et al.: The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest* 1997;111:106-109.
39. Naylor B: The pathognomonic cytologic picture of rheumatoid pleuritis. *Acta Cytol* 1990;34:465-473.
40. Zuffery P, Ruzicka J, Gerster JC: Pleural fluid cytology as an indication of an effusion of rheumatoid origin [Letter]. *J Rheumatol* 1993;20:1449-1451.
41. Naylor B: Cytological aspects of pleural, peritoneal, and pericardial fluids from patients with systemic lupus erythematosus. *Cytopathology* 1992;3:1-8.
42. Balachandran I, Jones DB, Humphrey DM: A case of *Pneumocystis carinii* in pleural fluid with cytologic, histologic, and ultrastructural documentation. *Acta Cytol* 1990;34:486-490.
43. Elwood LJ, Dobrzanski D, Feuerstein IM, Solomon D: *Pneumocystis carinii* in pleural fluid: The cytologic appearance. *Acta Cytol* 1991;35:761-764.
44. Magliocco A, Miller K, Robertson DI, Gill MJ: Occurrence of *Pneumocystis carinii* organisms in a peritoneal effusion from a patient with the acquired immunodeficiency syndrome. *Diagn Cytopathol* 1991;7:540-542.
45. Monte SA, Ehya H, Lang WR: Positive effusion cytology as the initial presentation of malignancy. *Acta Cytol* 1987;31:448-452.
46. Sears D, Hajdu SI: The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. *Acta Cytol* 1987;31:85-97.
47. Ringenberg QS, Doll DC, Loy TS, Yarbrow JW: Malignant ascites of unknown origin. *Cancer* 1989;64:753-755.
48. Gushchin V, Demmy TL, Kane JM 3rd: Surgical management of metastatic peritoneal or pleural disease. *Semin Oncol* 2007;34(3):215-225.
49. Price BA, Ehya H, Lee JH: Significance of pericellular lacunae in cell blocks of effusions. *Acta Cytol* 1992;36:333-337.
50. Ehya H: The cytologic diagnosis of mesothelioma. *Semin Diagn Pathol* 1986;3(3):196-203.
51. Kho-Duffin J, Tao LC, Cramer H, et al.: Cytologic diagnosis of malignant mesothelioma, with particular emphasis on the epithelial noncohesive cell type. *Diagn Cytopathol* 1999;20(2):57-62.
52. Sherman ME, Mark EJ: Effusion cytology in the diagnosis of malignant epithelioid and biphasic pleural mesothelioma. *Arch Pathol Lab Med* 1990;114:845-851.
53. Saad RS, Cho P, Liu YL, Silverman JF: The value of epithelial membrane antigen expression in separating benign mesothelial proliferation from malignant mesothelioma: A comparative study. *Diagn Cytopathol* 2005;32(3):156-159.
54. Dejmeek A, Hjerpe A: Reactivity of six antibodies in effusions of mesothelioma, adenocarcinoma and mesotheliosis stepwise logistic regression analysis. *Cytopathology* 2000;11(1):8-17.
55. Cury PM, Butcher DN, Corrin B, Nicholson AG: The use of histological and immunohistochemical markers to distinguish pleural malignant mesothelioma and in situ mesothelioma from reactive mesothelial hyperplasia and reactive pleural fibrosis. *J Pathol* 1999;189(2):251-257.

56. Aerts JG, Delahaye M, van der Kwast TH, et al.: The high post-test probability of a cytological examination renders further investigations to establish a diagnosis of epithelial malignant pleural mesothelioma redundant. *Diagn Cytopathol* 2006;34(8):523-527.
57. Roberts F, Harper CM, Downie I, Burnett RA: Immunohistochemical analysis still has a limited role in the diagnosis of malignant mesothelioma. A study of thirteen antibodies. *Am J Clin Pathol* 2001;116(2):253-262.
58. Attanoos RL, Griffin A, Gibbs AR: The use of immunohistochemistry in distinguishing reactive from neoplastic mesothelium. A novel use for desmin and comparative evaluation with epithelial membrane antigen, p53, platelet-derived growth factor-receptor, P-glycoprotein and Bcl-2. *Histopathology* 2003;43(3):231-238.
59. Xiao S, Renshaw A, Cibas ES, et al.: Novel fluorescence in situ hybridization approaches in solid tumors: Characterization of frozen specimens, touch preparations, and cytological preparations. *Am J Pathol* 1995;147:896-904.
60. Chhieng DC, Yee H, Schaefer D, et al.: Calretinin staining pattern aids in the differentiation of mesothelioma from adenocarcinoma in serous effusions. *Cancer* 2000;90:194-200.
61. Wiczkorek TJ, Krane JF: Diagnostic utility of calretinin immunohistochemistry in cytologic cell block preparations. *Cancer* 2000;90:312-319.
62. Hecht JL, Lee BH, Pinkus JL, Pinkus GS: The value of Wilms tumor susceptibility gene 1 in cytologic preparations as a marker for malignant mesothelioma. *Cancer* 2002;96:105-109.
63. Ordonez NG: Value of thyroid transcription factor-1, E-cadherin, BG8, WT1, and CD44S immunostaining in distinguishing epithelial pleural mesothelioma from pulmonary and non-pulmonary adenocarcinoma. *Am J Surg Pathol* 2000;24:598-606.
64. Cibas ES, Corson JM, Pinkus GS: The distinction of adenocarcinoma from malignant mesothelioma in cell blocks of effusions: the role of routine mucin histochemistry and immunohistochemical assessment of carcinoembryonic antigen, keratin proteins, epithelial membrane antigen, and milk fat globule-derived antigen. *Hum Pathol* 1987;18:67-74.
65. Frisman DM, McCarthy WF, Schlieff P, et al.: Immunocytochemistry in the differential diagnosis of effusions: use of logistic regression to select a panel of antibodies to distinguish adenocarcinomas from mesothelial proliferations. *Mod Pathol* 1993;6:179-184.
66. Ordonez NG: What are the current best immunohistochemical markers for the diagnosis of epithelioid mesothelioma? A review and update. *Hum Pathol* 2007;38(1):1-16.
67. Hecht JL, Pinkus JL, Weinstein LJ, Pinkus GS: The value of thyroid transcription factor-1 in cytologic preparations as a marker for metastatic adenocarcinoma of lung origin. *Am J Clin Pathol* 2001;116:483-488.
68. MacDougall DB, Wang SE, Zidar BL: Mucin-positive epithelial mesothelioma. *Arch Pathol Lab Med* 1992;116:874-880.
69. Zhang PJ, Livolsi VA, Brooks JJ: Malignant epithelioid vascular tumors of the pleura: Report of a series and literature review. *Hum Pathol* 2000;31:29-34.
70. Jaffe ES, Harris NL, Stein M, Vardiman JW (eds): *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours and Haematopoietic and Lymphoid Tissues*. Lyon, France, IARC Press, 2001.
71. Brimo F, Michel RP, Khetani K, Auger M: Primary effusion lymphoma a series of 4 cases and review of the literature with emphasis on cytomorphologic and immunocytochemical differential diagnosis. *Cancer* 2007;111(4):224-233.
72. Wakely PE Jr, Menezes G, Nuovo GJ: Primary effusion lymphoma: cytopathologic diagnosis using in situ molecular genetic analysis for human herpesvirus 8. *Mod Pathol* 2002;15(9):944-950.
73. Ansari MQ, Dawson DB, Nador R, et al.: Primary body cavity-based AIDS-related lymphomas. *Am J Clin Pathol* 1996;105:221-229.
74. Banks PM, Warnke RA: Primary effusion lymphoma. In Jaffe ES, Harris NL, Stein M, Vardiman JW (eds): *World Health Organization Classification of Tumours Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France, IARC Press, 2001, pp. 179-180.
75. Ohori NP, Whisnant RE, Nalesnik MA, Swerdlow SH: Primary pleural effusion posttransplant lymphoproliferative disorder: Distinction from secondary involvement and effusion lymphoma. *Diagn Cytopathol* 2001;25(1):50-53.
76. Renshaw AA, Nappi D, Cibas ES: Cytology of metastatic adenocarcinoma of the prostate in pleural effusions. *Diagn Cytopathol* 1996;15(2):103-107.
77. Parwani AV, Chan TY, Ali SZ: Significance of psammoma bodies in serous cavity fluid: A cytopathologic analysis. *Cancer* 2004;102(2):87-91.
78. Smith-Purslow MJ, Kini SR, Naylor B: Cells of squamous cell carcinoma in pleural, peritoneal, and pericardial fluids: Origin and morphology. *Acta Cytol* 1989;33:245-253.
79. Chhieng DC, Ko EC, Yee HT, et al.: Malignant pleural effusions due to small-cell lung carcinoma: A cytologic and immunocytochemical study. *Diagn Cytopathol* 2001;25(6):356-360.
80. Walts AE: Malignant melanoma in effusions: A source of false-negative cytodiagnoses. *Diagn Cytopathol* 1986;2:150-153.
81. Das DK: Serous effusions in malignant lymphomas: a review. *Diagn Cytopathol* 2006;34(5):335-347.
82. Walts AE, Marchevsky AM: Computerized interactive morphometry: An expert system for the diagnosis of lymphoid-rich effusions. *Am J Clin Pathol* 1989;92:765-772.
83. Billingham ME, Rawlinson DG, Berry PF, Kempson RL: The cytodiagnosis of malignant lymphomas and Hodgkin's disease in cerebrospinal, pleural, and ascitic fluids. *Acta Cytol* 1975;19:547-556.
84. Sasser RL, Yam YT, Li CY: Myeloma with involvement of the serous cavities: Cytologic and immunochemical diagnosis and literature review. *Acta Cytol* 1990;34:479-485.

Peritoneal Washings

EDMUND S. CIBAS

SPECIMEN COLLECTION, PREPARATION, AND REPORTING TERMINOLOGY

ACCURACY

THE NORMAL PERITONEAL WASHING

BENIGN CONDITIONS

Endosalpingiosis and Similar Benign Proliferations
Endometriosis

MALIGNANT TUMORS

Ovarian Cancer
Endometrial Cancer
Cervical Cancer
Other Malignancies

MONITORING RESPONSE TO TREATMENT ("SECOND-LOOK PROCEDURES")

Peritoneal washing cytology (PWC) was introduced in the 1950s as a way to identify microscopic spread of cancer not visible by gross inspection of the peritoneal surface.¹ In some cancer patients, positive PWC may be the only evidence of metastatic disease to the peritoneum. Because positive results correlate with a poorer prognosis,^{2,3} cytologic findings are included in the staging systems for ovarian,⁴ fallopian tube,⁵ and endometrial cancers.⁶ The yield, however, is low; positive washings by themselves change the surgical stage of only 3% to 5% of women with gynecologic cancers.^{7,8}

PWC is also used to exclude an occult cancer in patients who undergo laparoscopy or laparotomy for presumed benign gynecologic conditions, such as endometriosis or leiomyomata. Cytologic findings can be employed to monitor a patient's response to treatment for advanced ovarian cancer and other malignancies (the "second-look" procedure), but this is usually limited to patients in research protocols.⁹⁻¹³ In some instances, PWC is used to detect peritoneal spread of nongynecologic malignancies, such as pancreatic and gastric cancer.

Indications for peritoneal washings (summary):

- staging gynecologic malignancies
 - ovarian
 - fallopian tube
 - endometrial
- ruling out occult cancer
- assessing response to treatment (the "second-look" procedure)
- staging nongynecologic malignancies
 - pancreatic
 - gastric

SPECIMEN COLLECTION, PREPARATION, AND REPORTING TERMINOLOGY

On entering the peritoneal cavity, the surgeon evacuates any preexisting peritoneal fluid and submits it separately for cytologic examination. Washings are obtained by instilling 50 to 200 mL of sterile saline or other physiologic solution into several different areas, usually the pelvis, the right and left paracolic gutters, and the undersurface of the diaphragm. The fluid is aspirated, and heparin is added to prevent clotting. Segregating washings from different sites does not have any advantage over combining them into a single specimen.¹⁴

The specimen should be delivered to the laboratory unfixed and refrigerated at 4°C until slides can be prepared. If a significant delay before cytopreparation is anticipated, an equal volume of 50% ethanol can be added. To prepare slides, the specimen is thoroughly mixed, and an aliquot (often 50 mL) is centrifuged to a cell sediment/pellet. From this sediment one can prepare direct smears, filter preparations, cytocentrifuge preparations, or thinlayer preparations, depending on the resources of the laboratory.¹⁵ The remaining (or separately centrifuged) cell sediment can also be fixed in 10% formalin, embedded in paraffin, and processed as a histologic specimen ("cell block"). Cell block sections are quite useful, especially for morphologic comparison to the patient's resected neoplasm.¹⁶

Results of PWC are commonly reported as "negative," "atypical," "suspicious," or "positive."¹⁷ Atypical

and suspicious diagnoses, however, should be avoided whenever possible because they are not helpful to a physician faced with making a treatment decision.^{15,18} In most cases, only an unequivocally positive diagnosis is used for staging purposes—anything less is treated as a negative result. Side-by-side comparison with the corresponding resection specimen often helps resolve an equivocal case.

Criteria for specimen adequacy have not been established, but it seems reasonable to require some benign mesothelial cells before considering a peritoneal washing specimen adequate and negative for malignant cells.¹⁹ A specimen with malignant cells is always adequate.

Because peritoneal washings are obtained as part of a cancer staging procedure, there is usually a concurrent histologic specimen. For example, when washings are obtained as part of ovarian cancer staging, an oophorectomy is obtained by the laboratory at the same time. Representative slides from the oophorectomy specimen can be helpful in a side-by-side comparison with a diagnostically difficult washing specimen. Similarly, when washings are obtained as part of an endometrial staging procedure, representative sections from the hysterectomy can be useful in resolving any difficulty encountered in the evaluation of the washings. With endometrial cancers, unlike ovarian cancers, there is a prior positive endometrial biopsy or curettage report that can also provide useful information regarding the histologic subtype and grade.

ACCURACY

Not all patients with metastases to the peritoneum have positive PWC results. In fact, 23% to 52% of patients with biopsy-proven peritoneal involvement have negative results.^{17,20-23} When peritoneal washings are examined as part of a second-look procedure, the false-negative rate is even higher, ranging from 31% to 86% of patients with biopsy-proven metastases.^{9,11,21,22,24-26} The higher false-negative rate of second-look procedures may be as a result of the poor distribution of fluid when the abdominal cavity is altered by adhesions.

False-positive diagnoses are uncommon but well documented,^{2,3,21,27-29} occurring in less than 5% of cases.^{2,23,30} Causes include reactive mesothelial proliferation with psammoma bodies^{23,27,28} and endometriosis.^{22,29} In particular, eosinophilic metaplastic atypia in endometriosis can be a source of atypical (but benign) cells that might be misconstrued as malignant.³¹ In one instance, ectopic pancreatic tissue was misinterpreted as adenocarcinoma in a patient with a mucinous ovarian cancer.³²

THE NORMAL PERITONEAL WASHING

Peritoneal washings differ morphologically from peritoneal (ascitic) fluids in several easily recognizable ways. First, the washing procedure mechanically strips the peritoneal surface of entire sheets of mesothelial cells, whereas sheets of mesothelial cells are not seen in a benign ascitic fluid. Second, skeletal muscle and adipose tissue fragments are common in peritoneal washings, especially in cell block preparations.

CYTOMORPHOLOGY OF BENIGN PERITONEAL WASHINGS:

- mesothelial cells in sheets
- collagen balls
- histiocytes
- skeletal muscle
- adipose tissue

Mesothelial cells in peritoneal washings are arranged predominantly in flat sheets, often large and occasionally folded (Fig. 5.1). In cell block sections the sheets are transected and appear as long, thin ribbons (Fig. 5.2). The cells are evenly spaced, with a moderate amount of cytoplasm. Nuclear membranes are thin, and the chromatin is pale and evenly dispersed; small nucleoli are often present. Although usually round or oval (Fig. 5.3), the nuclei are sometimes scalloped, wrinkled, or grooved,³³ possibly as a result of fixation artifact. Isolated mesothelial cells, similar to those seen in ascites, are also encountered.

Spherical masses of collagen surrounded by benign, flattened mesothelial cells, known as *collagen balls*, are seen in up to 50% of peritoneal washings.^{33,34} Aqua in color with the Papanicolaou stain, they have round or bosselated contours (Fig. 5.4). They are usually few in number, but occasionally they can be abundant. They have no known significance (and therefore do not deserve mention on a cytology report). It has been suggested that they result from a pinching off of mesothelial-lined stromal projections known as “micropapillomatosis” on the surface of the ovaries.^{34,35}

Numerous *histiocytes*, scattered either as isolated cells or in variably-sized aggregates, are often present (Fig. 5.5). Because they look different from mesothelial cells and are often haphazardly aggregated, they might be misinterpreted as metastatic cancer cells. Attention to the typical nuclear and cytoplasmic features of histiocytes (oval, folded, and kidney-shaped nuclei; pale chromatin; granular and microvacuolated cytoplasm) is helpful in correctly identifying them. *Skeletal muscle* and

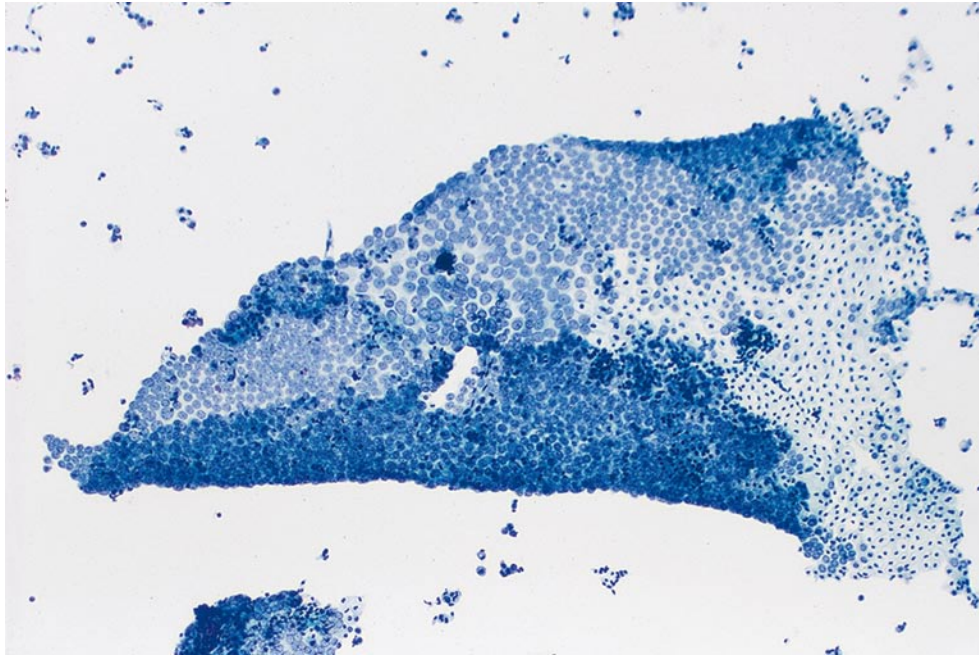


Figure 5.1 Mesothelial cells. Normal mesothelial cells in peritoneal washings are often arranged in cohesive sheets. Although the sheets are flat, they can be folded or rolled on cytocentrifuge or thinlayer preparations. Isolated mesothelial cells are present in the background (Papanicolaou stain).

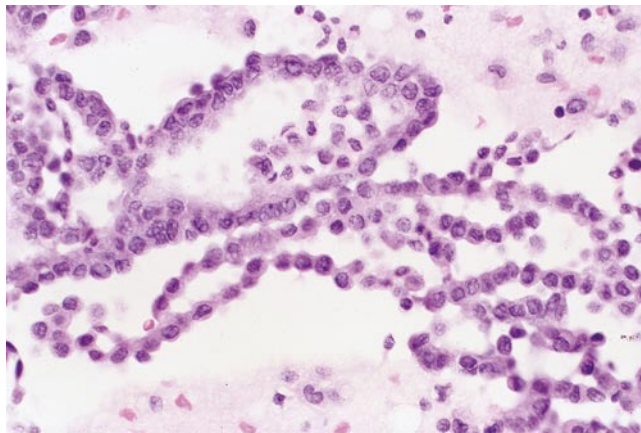


Figure 5.2 Mesothelial cells (cell block). In cell block sections, a sheet of mesothelial cells is transected and looks like a string of pearls. When it curls around on itself it has a pseudo-glandular appearance (hematoxylin-eosin [H & E] stain).

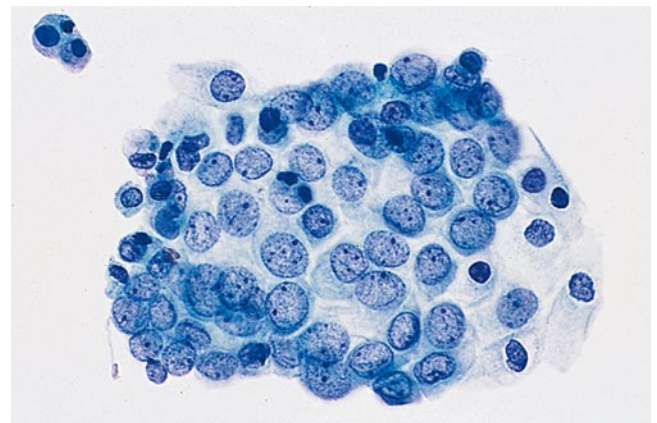


Figure 5.3 Mesothelial cells. Usually, benign mesothelial cells have round or oval nuclei, but in some cases there are irregularities in nuclear contour. Note the spaces ("windows") that separate adjacent cells, a common finding (Papanicolaou stain).

adipose tissue fragments are sometimes seen. (They fall into the peritoneal cavity when the abdominal incision is made and are suctioned along with the fluid.) *Detached ciliary tufts*, presumably of fallopian tube origin, are a relatively common incidental finding, especially when

the washings are obtained during the secretory (luteal) phase of the menstrual cycle.³⁶

A typical interpretation of a benign peritoneal washing might read as follows: "**Negative for malignant cells.** Mesothelial cells and histiocytes."

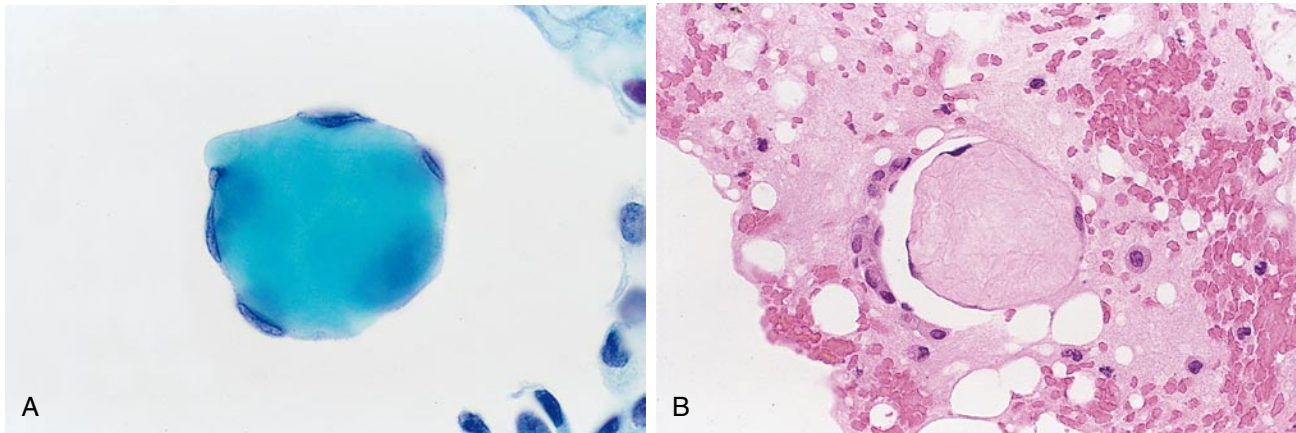


Figure 5.4 Collagen ball. These spheres of collagen are surrounded by flattened mesothelial cells. A, Papanicolaou stain; B, hematoxylin-eosin (H & E)-stained cell block section.

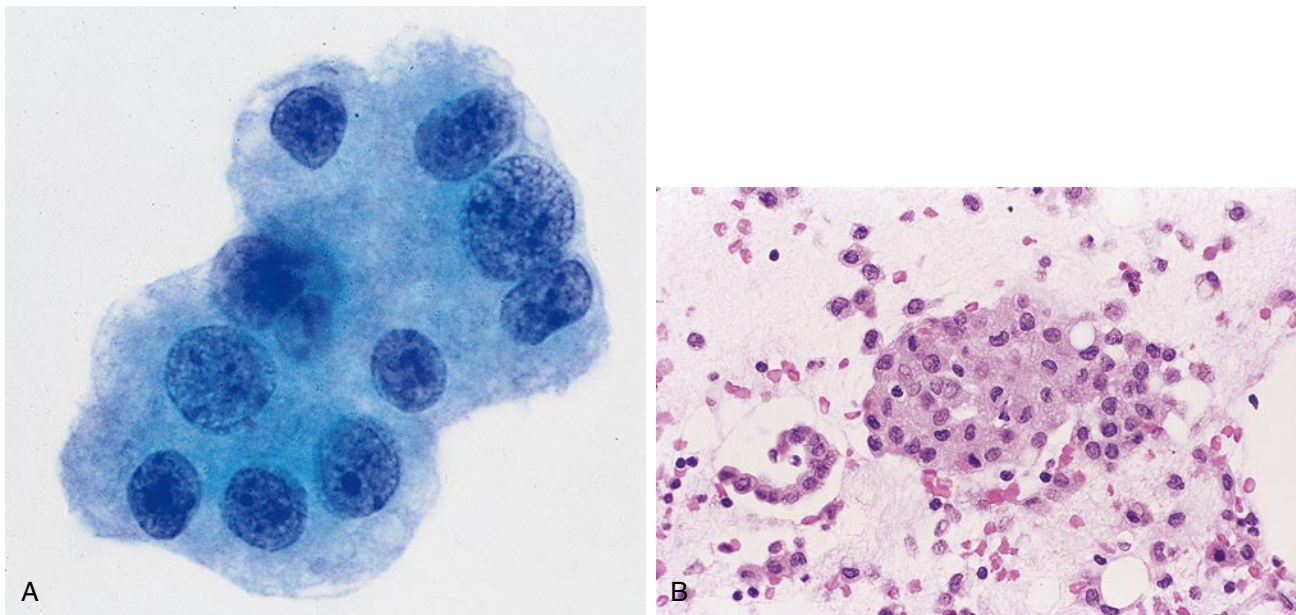


Figure 5.5 Histiocytes. Histiocytes are often in groups, as seen here. They have indistinct cell borders (no “windows”), granular or vacuolated cytoplasm, and occasionally folded nuclei. A short strip of mesothelial cells is also present on the cell block section. A, Papanicolaou stain; B, hematoxylin-eosin (H & E)-stained cell block.

BENIGN CONDITIONS

Endosalpingiosis and Similar Benign Proliferations

A group of benign conditions involving the peritoneal and ovarian surfaces can result in a proliferation of fallopian tubal-type epithelial cells or mesothelial cells, often with psammoma bodies. These conditions mimic peritoneal involvement by a serous borderline tumor and serous adenocarcinoma; familiarity with them is thus important.

Endosalpingiosis is a proliferation of benign glands and cysts lined by ciliated, fallopian tubelike epithelium. Common locations include the ovarian cortex, uterine serosa, peritoneal surface, and omentum. Psammoma bodies are often seen. Some authors reserve the term *endosalpingiosis* for proliferations that include tubal type

stroma and glands, and use the term *Mullerian inclusion cysts* for cases that lack tubal-type stroma.³⁵ Both endosalpingiosis and Mullerian inclusion cysts, however, are usually incidental, microscopic findings in a patient undergoing surgery for something else. Histologically, they raise the possibility of metastatic disease but are identified as benign proliferations because the cells are uniform and bland and lack mitotic activity.

Serous adenofibromas of the ovarian surface are benign ovarian tumors that, like endosalpingiosis, are comprised of benign tubal-type glands or cysts, except that they are often a visible mass rather than a microscopic finding, and they have a broad fibrous stromal component. The epithelial portion of a serous adenofibroma, like endosalpingiosis, often contains psammoma bodies and can be confused with peritoneal involvement by a serous borderline tumor or even adenocarcinoma.

Prior surgery, pelvic inflammatory disease, a ruptured cyst, and other conditions cause *florid mesothelial hyperplasia*, sometimes accompanied by psammoma body formation.¹⁵

CYTOMORPHOLOGY OF ENDOSALPINGIOSIS AND SIMILAR BENIGN PROLIFERATIONS:

- cuboidal cells ($\pm$ cilia) with minimal-to-mild atypia
- psammoma bodies

The conditions previously described all have a similar appearance in peritoneal washings. Clusters of cuboidal mesothelial-like cells (Fig. 5.6), some arranged around a psammoma body (Figs. 5.7, 5.8) or nondescript calcification, are present and can be abundant.²⁸ Cilia may be present, but often they are absent or impossible to

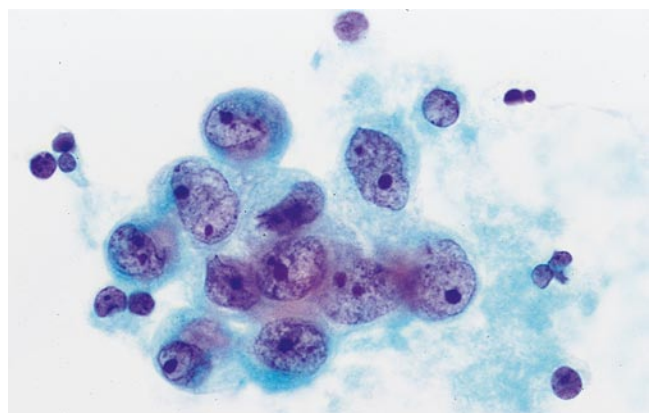


Figure 5.6 Reactive mesothelial cells (ovarian torsion). The mesothelial cells are enlarged, with irregular nuclei and prominent nucleoli. Exploratory laparotomy revealed torsion of the right ovary with intraperitoneal adhesions.

identify. Nuclei are round or oval, with a pale chromatin pattern and small nucleoli. Nuclear atypia is mild, and mitoses are uncommon.

These benign conditions are a potential cause of a false-positive interpretation of involvement by a serous adenocarcinoma or borderline tumor.²⁷ To avoid a false-positive interpretation, a diagnosis of malignancy must not be based on the presence of psammoma bodies alone.^{22,27} Correlation with the concurrent histologic material is helpful in most cases. Certainly, if the patient does not have a serous adenocarcinoma or borderline tumor, one should avoid rendering such a diagnosis on the basis of the peritoneal washings alone. Some patients with endosalpingiosis or benign mesothelial proliferations, however, can also have a serous borderline tumor confined to the ovary.¹⁸ Comparison of the peritoneal washings, particularly cell block preparations, with the histologic sections from the tumor often resolves equivocal cases, but the final interpretation is not always straightforward, and the pathologist is sometimes required to make the best judgment they can. There are few helpful morphologic clues, particularly because many serous borderline tumors have only mild cytologic atypia, equivalent to what can be seen with florid mesothelial hyperplasia and endosalpingiosis. One possible clue: in cell block sections, some papillary fragments of serous borderline tumors have broad stromal cores, a feature not seen in cell blocks containing only mesothelial hyperplasia or endosalpingiosis.

Endometriosis

Endometriosis, the presence of ectopic benign endometrial glands and stroma in the omentum, peritoneum, ovary, and elsewhere, is another pitfall in the evaluation of peritoneal washings.^{22,29}

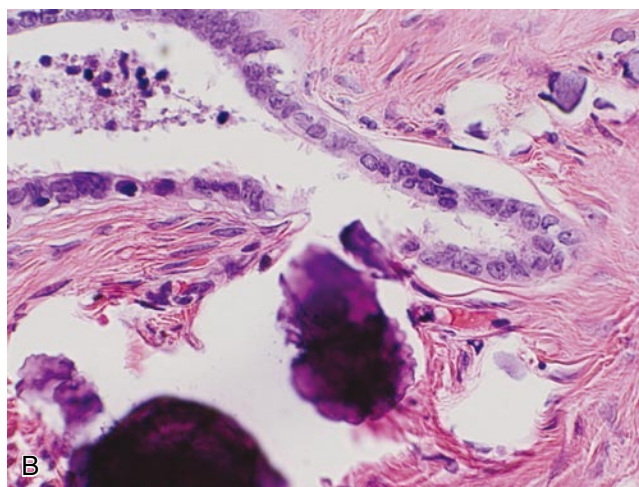
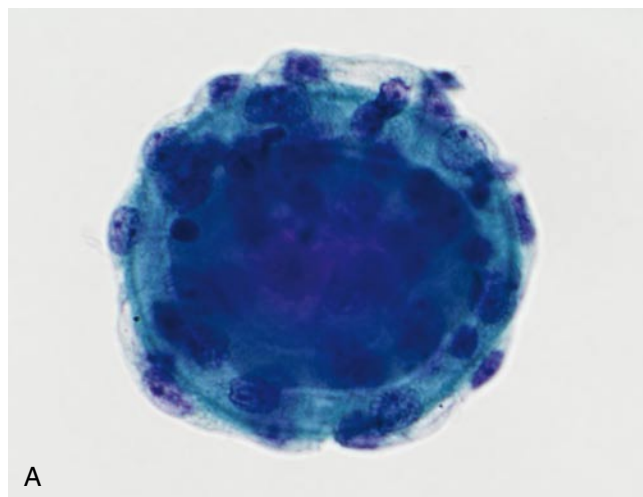


Figure 5.7 Endosalpingiosis. A, The psammoma body has concentric lamination and is surrounded by cuboidal cells with small nucleoli and vacuolated cytoplasm. Cilia are not identified (Papanicolaou stain). B, The surface of the resected ovary shows benign ciliated glandular cells associated with psammoma bodies. There was no evidence of tumor (hematoxylin-eosin [H & E] stain).

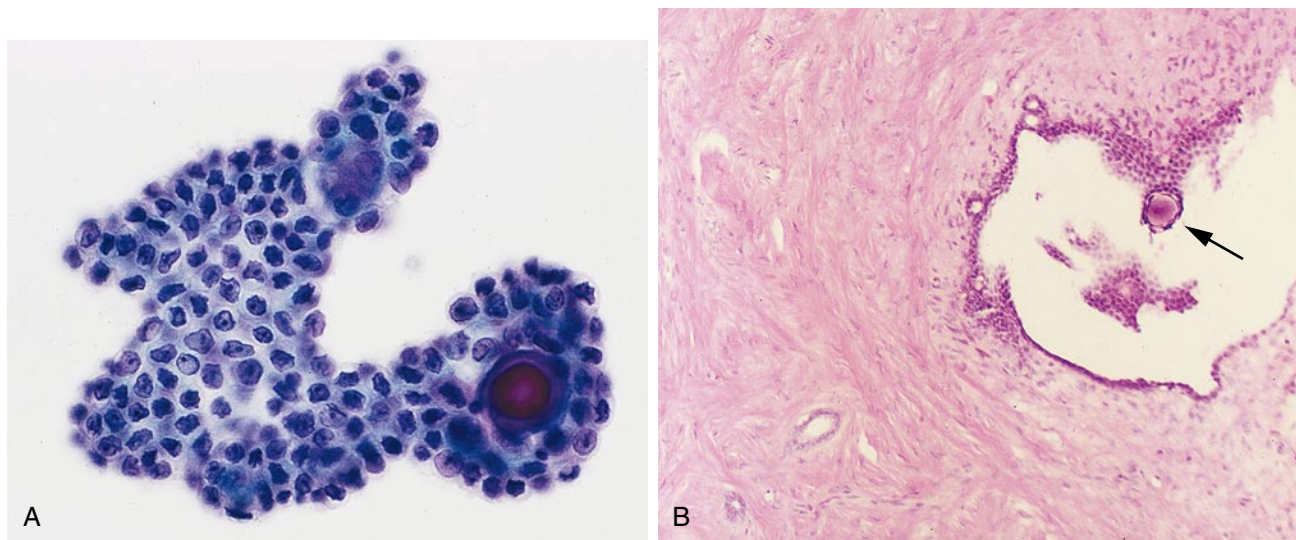


Figure 5.8 Serous adenofibroma of the ovary. A, A psammoma body is surrounded by evenly spaced mesothelial cells with minimal nuclear atypia (Papanicolaou stain). B, The resected ovary shows a serous adenofibroma involving the ovarian surface, with an occasional psammoma body (arrow; hematoxylin-eosin [H & E stain]).

CYTOMORPHOLOGY OF ENDOMETRIOSIS:

- hemosiderin-laden macrophages
- endometrial glandular cells
- endometrial stromal cells
- tissue fragments containing endometrial glands and stroma (cell block)

The purpose of peritoneal washings in this setting is to exclude an occult malignancy, not to diagnose endometriosis. A diagnosis of endometriosis, in fact, can rarely be made by examining peritoneal washings alone. Hemosiderin-laden macrophages are seen in one third of women with endometriosis.³⁷ In a young woman, hemosiderin-laden macrophages are most likely the result of endometriosis,³⁷ but by themselves they are a nonspecific finding seen in any condition associated with intraperitoneal bleeding. Some cases of endometriosis show a population of cells morphologically similar to but distinct (in subtle ways) from mesothelial cells and macrophages; these likely represent endometrial glandular or stromal cells. The endometrial-like glandular and stromal cells may, in fact, resemble clusters of exfoliated endometrial cells as seen in cervical-vaginal preparations (Fig. 5.9A and B), but a definitive distinction between mesothelial cells and endometrial cells is extremely difficult.³⁷ On rare occasions, a diagnosis can be made when tissue fragments with endometrial-type glands and stroma are identified in cell block sections (Fig. 5.9B and C).

Occasionally, the endometrial glandular cells are enlarged and atypical and might suggest a malignancy. Correlation with concurrent histologic material can be quite helpful in preventing a misdiagnosis.^{29,31}

MALIGNANT TUMORS

Ovarian Cancer

Ovarian cancer is the seventh most common cancer in women in the United States and accounts for 6% of cancer-related mortality.³⁸ The median age at diagnosis is 52 years. Most women (70% to 75%) with ovarian cancer present with stage III or IV disease (tumor spread beyond the pelvis). PWC is an important staging component; a positive finding modifies stage I and II tumors to stage Ic and IIc, respectively (Table 5.1). Primary treatment for presumed ovarian cancer is surgical staging and cytoreduction. Most women (except those with stage IA and IB, grade 1 tumors) receive postoperative chemotherapy, either systemic or intraperitoneal, depending on the stage of their disease. Platinum drugs (cisplatin, carboplatin) and taxanes (paclitaxel, docetaxel) are the most efficacious and are often used in combination.

The most common type of ovarian cancer—and the type that most often produces positive cytologic findings—is the *serous adenocarcinoma*.

CYTOMORPHOLOGY OF SEROUS ADENOCARCINOMA:

- large or small clusters and isolated cells
- large cells
- marked variation in nuclear size
- nuclear hyperchromasia
- prominent nucleoli
- mitoses
- scant or abundant vacuolated cytoplasm

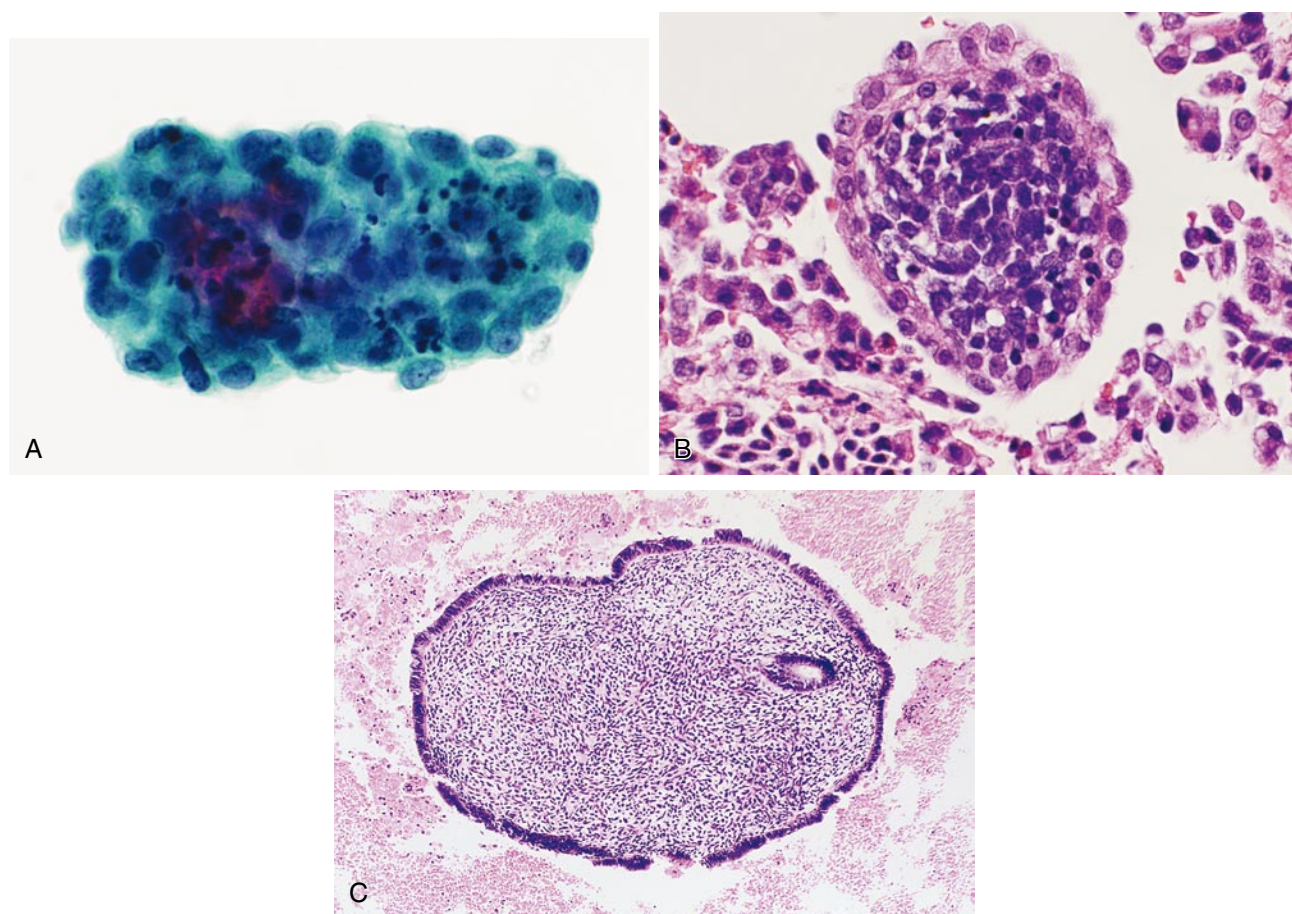


Figure 5.9 Endometriosis. A, A cluster of cells has scalloped borders and contains nuclear fragments. Individually, the cells resemble mesothelial cells, but the nuclear debris suggests that they are degenerating endometrial cells. Such clusters are often recognized as endometrial cells only in retrospect, after reviewing cell block sections or correlating with laparoscopic or histologic evidence. B, The cell block from the same case as A shows endometrial glands surrounding a core of degenerated endometrial stromal cells. C, Occasionally, the cell block of peritoneal washings from a patient with endometriosis shows diagnostic tissue fragments composed of intact endometrial-type glands and stroma (hematoxylin-eosin [H & E] stain).

TABLE 5.1 DEFINITIONS OF THE STAGES IN PRIMARY CARCINOMA OF THE OVARY

Stage I	Growth limited to the ovaries
Stage Ia	Growth limited to one ovary; no ascites; no tumor on the external surface; capsule intact
Stage Ib	Growth limited to both ovaries; no ascites; no tumor on the external surfaces; capsules intact
Stage Ic	Tumor either stage Ia or Ib, but with tumor on surface of one or both ovaries; or with capsule ruptured; or with ascites present containing malignant cells or with positive peritoneal washings
Stage II	Growth involving one or both ovaries with pelvic extension
Stage IIa	Extension or metastases to the uterus or tubes
Stage IIb	Extension to other pelvic tissues
Stage IIc	Tumor either stage IIa or IIb, but with tumor on surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with positive peritoneal washings
Stage III	Tumor involving one or both ovaries with peritoneal implants outside the pelvis or positive retroperitoneal or inguinal nodes. Superficial liver metastasis equals stage III. Tumor is limited to the true pelvis but with histologically proven malignant extension to small bowel or omentum
Stage IIIa	Tumor grossly limited to the true pelvis with negative nodes but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces
Stage IIIb	Tumor involving one or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter. Nodes are negative
Stage IIIc	Abdominal implants greater than 2 cm in diameter or positive retroperitoneal or inguinal nodes
Stage IV	Growth involving one or both ovaries with distant metastases. If pleural effusion is present, there must be positive cytology to allot a case to stage IV

From FIGO Cancer Committee: Staging announcement. *Gynecol Oncol* 1986;25:383-385.

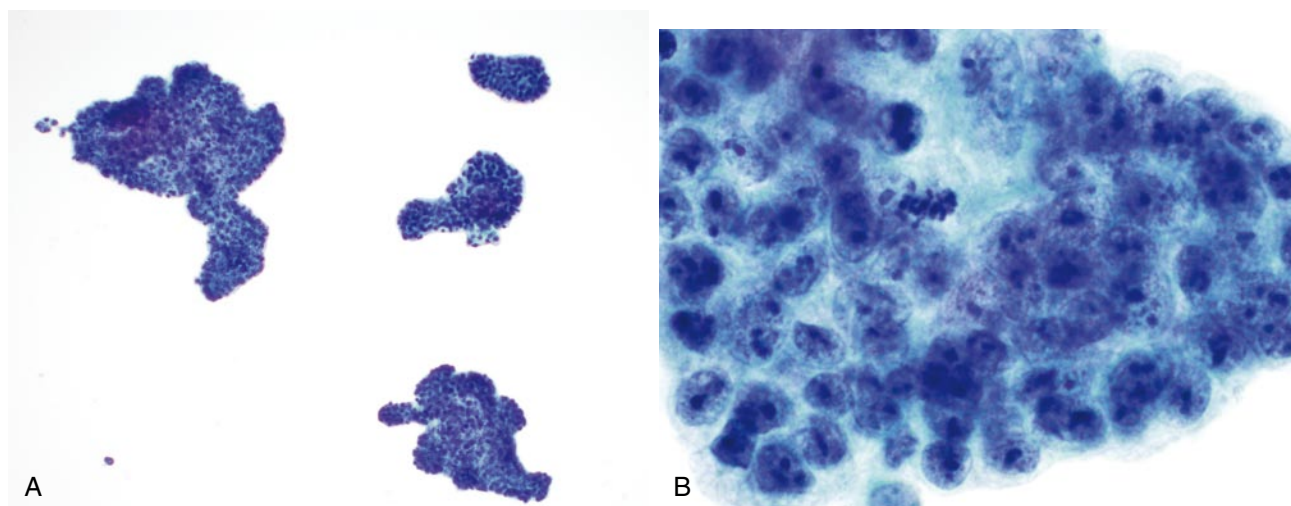


Figure 5.10 Serous adenocarcinoma of the ovary. A, The malignant cells of serous carcinomas are often arranged in large, irregularly shaped three-dimensional clusters of crowded cells (Papanicolaou stain). B, Higher magnification reveals crowded large cells with round, dark nuclei, large nucleoli, and pale vacuolated cytoplasm. Mitoses are often present (Papanicolaou stain).

Serous adenocarcinoma is usually easy to recognize in peritoneal washings. The malignant cells are isolated and arranged in clusters (Fig. 5.10). Nuclei are enlarged and vary markedly in size. There is nuclear hyperchromasia, with coarsely textured chromatin and prominent nucleoli (Fig. 5.11). Mitoses are common. Cytoplasm may be scant but is often abundantly vacuolated. Psammoma bodies may be present. Morphologically identical tumors occur as primary neoplasms of the peritoneum (see Fig. 5.11).

Serous borderline tumor is a low-grade neoplasm with a significantly better prognosis than the serous adenocarcinoma (87% versus 50% 5-year survival rate, respectively).¹² The distinction is based on the presence or absence of stromal invasion within the primary tumor. Although the distinction cannot be made on the basis of

peritoneal washings, some differences between the two diseases are apparent cytologically.^{33,39}

CYTOMORPHOLOGY OF SEROUS BORDERLINE TUMOR:

- large or small clusters and isolated cells
- minimal-mild nuclear atypia
- cytoplasmic vacuoles
- psammoma bodies
- fibrovascular cores (cell block)

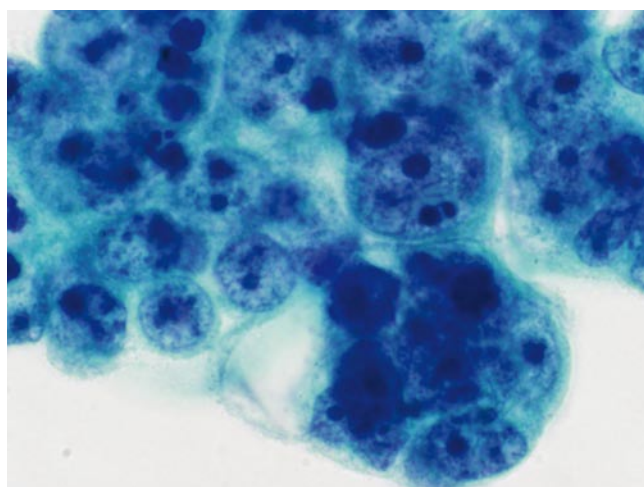


Figure 5.11 Serous adenocarcinoma of the peritoneum. These tumors are morphologically indistinguishable from serous carcinomas of the ovary (Papanicolaou stain).

Positive peritoneal washings from patients with serous borderline tumors usually show large (Fig. 5.12A) or small (Fig. 5.12B), cohesive, three-dimensional, often branching, clusters. Isolated cells are less conspicuous than in serous adenocarcinoma. Cellular pleomorphism is slight or moderate. Cytoplasmic vacuoles can be seen, and the nuclear-to-cytoplasmic ratio is quite high. Nucleoli are inconspicuous, and mitoses are rare. As with serous adenocarcinomas, psammoma bodies may be present. Cell blocks sometimes show thin or broad fibrovascular cores lined by neoplastic epithelium.

The differential diagnosis, particularly for the serous borderline tumors, includes endosalpingiosis and similar benign proliferations. A malignant diagnosis should never be made solely on the basis of psammoma bodies. The evaluation of peritoneal washings in patients with serous borderline tumors depends on a careful comparison with the resected primary tumor. Cell block sections are especially useful in this regard. Broad fibrovascular cores lined by atypical epithelium are characteristic of serous borderline tumors and are rarely seen in patients with endosalpingiosis or mesothelial hyperplasia (Fig. 5.13).

Positive washings in a woman with a serous borderline tumor do not have the same clinical significance

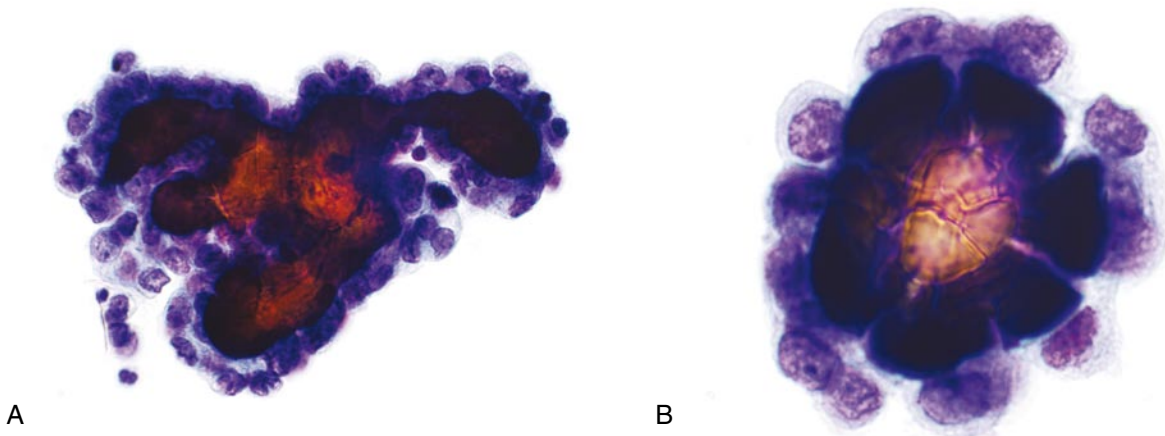


Figure 5.12 Serous borderline tumor. A, Some neoplastic cells exfoliate as large branching micropapillary clusters with smoothly contoured or slightly scalloped edges. The centers are occupied by microcalcifications (Papanicolaou stain). B, A smaller cluster of cuboidal cells surrounding a cracked psammoma body from the same case demonstrates the minimal atypia characteristic of the serous borderline tumor. Biopsies showed implants of identical cells on the omentum and peritoneum. Compare with Figure 5.7: the distinction between endosalpingiosis and a serous borderline tumor is often extremely difficult (Papanicolaou stain).

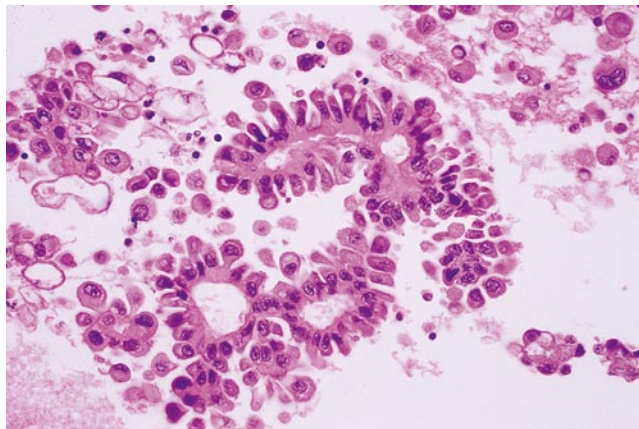


Figure 5.13 Serous borderline tumor. Cell block sections are helpful because they may show papillary structures lined by atypical cells with stratification and tufting, features that are characteristic of these tumors (hematoxylin-eosin [H & E] stain).

as in a woman with an adenocarcinoma. Postoperative chemotherapy offers no survival advantage in women with stage Ic or II serous borderline tumors, and the overall survival rate is good even without treatment. In fact, positive washings in women with borderline ovarian tumors raise a question regarding terminology. Because these tumors are not, strictly speaking, carcinomas, it is best to report positive washings in a woman with a borderline tumor as “**Neoplastic cells present. Consistent with involvement by the patient’s known serous borderline tumor.**” The traditional phrase *positive for malignant cells* should be avoided because it might imply that the cytologist is converting the borderline diagnosis (made after examination of the ovary) to a carcinoma diagnosis. This is not, of course, how the distinction between a borderline tumor and carcinoma is made, but nevertheless potentially confuses the oncologist.

Less common than the serous tumors are the *mucinous, endometrioid, clear cell, and transitional cell tumors of the ovary*. There is significant overlap in the morphologic features of these tumors when they appear in peritoneal washings, such that distinction among them is not reliable (nor is it necessary) based on PWC. It is sufficient to report results as “**Positive for malignant cells. Consistent with adenocarcinoma.**” Alternatively, if one holds the report of the washings until the concurrent ovarian tumor resection has been examined and subtyped (e.g., as a serous adenocarcinoma), one can report the washings as “**Positive for malignant cells. Consistent with serous adenocarcinoma.**”

A mucinous tumor in the ovary, particularly one associated with pseudomyxoma peritonei, is often a metastasis (the appendix is a likely primary site) rather than a primary ovarian neoplasm (Fig. 5.14). Primary versus metastatic mucinous tumors of the ovary are difficult (often impossible) to distinguish by conventional histologic examination. Clinical findings and differential staining for cytokeratins CK7 and CK20 are often needed.

Germ cell (Fig. 5.15) and sex cord-stromal tumors of the ovaries can also involve peritoneal surfaces.⁴⁰

Endometrial Cancer

Unlike ovarian cancers, most endometrial cancers are detected at an early stage and are therefore potentially curable. In 1988 the International Federation of Gynecology and Obstetrics (FIGO) adopted a surgical staging system (Table 5.2) in which peritoneal washings play an important role; positive results signify stage IIIA disease.⁴¹

Malignant cells from an endometrial cancer can spread to the peritoneal cavity by direct extension through the myometrium and serosa, by vascular or

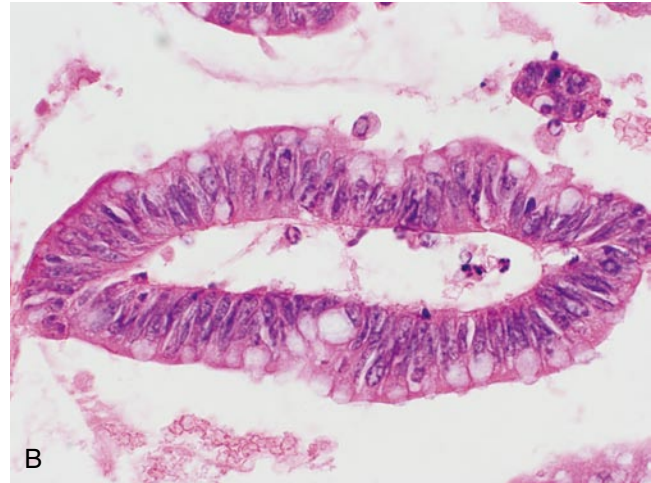


Figure 5.14 Mucinous adenocarcinoma of the appendix mimicking an ovarian mucinous adenocarcinoma. The tumor cells are columnar, with elongated nuclei and a high nuclear-to-cytoplasmic ratio. The patient presented with pseudomyxoma peritonei and was found to have bilateral ovarian metastases. A, Papanicolaou stain; B, hematoxylin-eosin (H & E)-stained cell block.

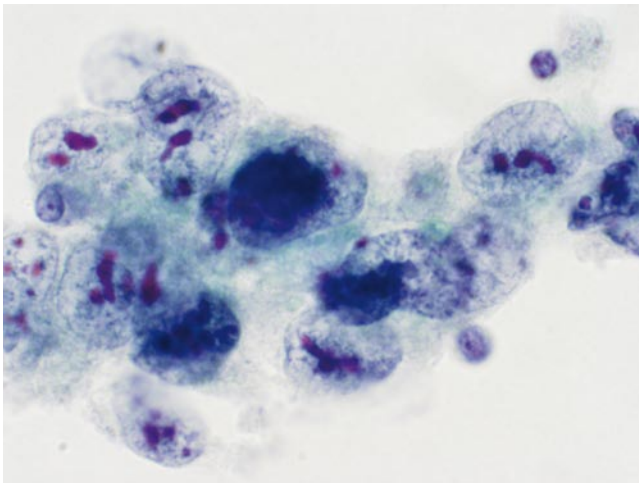


Figure 5.15 Embryonal carcinoma of the ovary. The cells are large and pleomorphic, with vesicular nuclei, prominent, irregular, and multiple nucleoli, and pale cytoplasm (Papanicolaou stain).

lymphatic invasion, or by retrograde migration through the fallopian tubes. After complete surgical staging, most women with endometrial cancer and positive PWC also have adnexal or lymph node involvement, and adjuvant therapy will be considered in such cases. (They are also more likely to have a positive cervical smear.⁴²) A small proportion (2% to 15%) of women with endometrial cancer limited to the uterus (and even to the endometrium) have positive PWC results.^{30,42-46} For this reason it is thought that, at least in some cases, endometrial cancer spreads to the peritoneum by retrograde migration through the fallopian tubes. Malignant cells are found in the lumina of the fallopian tubes in 41% of women with endometrial cancer.⁴⁷ Transtubal dissemination might be

TABLE 5.2 SURGICAL STAGING CRITERIA FOR UTERINE CORPUS CANCERS

Stage I	The tumor is confined to the uterine corpus.
Stage IA	The tumor is limited to the endometrium.
Stage IB	The tumor invades to half or less of the myometrial thickness.
Stage IC	The tumor invades to more than half of the myometrial thickness.
Stage II	The tumor extends to the cervix.
Stage IIA	The tumor involves endocervical glands only.
Stage IIB	The tumor involves cervical stroma.
Stage III	The tumor demonstrates regional spread.
Stage IIIA	The tumor involves serosa or adnexa or positive peritoneal cytology .
Stage IIIB	There are vaginal metastases.
Stage IIIC	The tumor metastasizes to pelvic or para-aortic lymph nodes.
Stage IV	Advanced pelvic or distant disease is present.
Stage IVA	The tumor invades bladder or bowel mucosa.
Stage IVB	There are distant metastases, including intra-abdominal sites or inguinal lymph nodes.

From FIGO Cancer Committee: Announcements. *Gynecol Oncol* 1989; 35:125-127.

facilitated by the prior endometrial biopsy or curettage, or by preoperative intracavitary placement of radium implants. Some have found that transtubal dissemination might also occur during diagnostic hysteroscopy, which involves distending the uterine cavity with fluid,⁴⁸ but others have found no evidence for this when lower pressures were used (<80 mm Hg).⁴⁹ Laparoscopically assisted vaginal hysterectomy, applied with increasing frequency in the management of women with endometrial cancer, requires a uterine manipulator that might also cause iatrogenic dissemination of endometrial cancer cells.⁵⁰ Some investigators, however, have found

no increase in positive PWC when certain manipulators were used.⁵¹ Clamping the fallopian tubes before hysteroscopy appears to prevent dissemination of malignant endometrial cells.⁵²

The most common histologic subtype of endometrial cancer, accounting for approximately 85% of cases, is the endometrioid type. These tumors range from well-differentiated adenocarcinomas (the most common) with abundant gland formation and only slight nuclear atypia, to poorly differentiated tumors with little or no glandular differentiation and marked nuclear pleomorphism.

CYTOMORPHOLOGY OF ENDOMETRIAL CANCER (ENDOMETRIOID):

- cell clusters and isolated cells
- enlarged nuclei
- coarse chromatin
- nuclear pleomorphism (high-grade tumors)
- scant or vacuolated cytoplasm

Peritoneal washings positive for the common *endometrioid type* of endometrial cancer show clusters and isolated malignant cells. Nuclei are round and enlarged, with coarsely textured chromatin and prominent nucleoli (Fig. 5.16). Cytoplasm is scant or moderate in amount. Nuclear pleomorphism is usually mild but can be marked in high-grade tumors. Cell block sections can be helpful by demonstrating tubular glands and squamous morule formation similar to that seen in histologic sections from the primary endometrial tumor (Fig. 5.17).

Some of the less common types of endometrial cancer, such as the *papillary serous* and *clear cell* types, are more aggressive, tending to spread to the peritoneum more often than the endometrioid type. These tumors have

a high nuclear grade and resemble high-grade ovarian cancers (Fig. 5.18). Malignant mixed müllerian tumors of the uterus can spread to the peritoneum. In most cases the washings demonstrate the adenocarcinoma component only, but they can contain both adenocarcinoma and sarcoma, or the sarcomatous component alone.^{53,54} As with the ovarian cancers, there is significant overlap in the morphologic features of the various endometrial cancer subtypes when they appear in peritoneal washings, such that distinction among them is not reliable (or necessary) based on PWC. It is sufficient to report results as “**Positive for malignant cells.** Consistent with adenocarcinoma.” Alternatively, if the concurrent endometrial tumor resection has been histologically classified (e.g., as a clear cell adenocarcinoma), one can report the washings as “**Positive for malignant cells.** Consistent with endometrial adenocarcinoma, clear cell type.”

The management of a woman with endometrial cancer and positive PWC is controversial. It is not clear whether positive cytologic findings alone indicate an increased risk for recurrence. Perhaps positive PWC, in the absence of other poor prognostic markers, merely represents a harmless dissemination of cells that are not even viable. This hypothesis has been challenged: exfoliated endometrial cancer cells, collected in run-off fluid through the fallopian tubes by flushing the uterus with saline after hysterectomy, do grow in culture in some cases, and thus appear to be functionally viable.⁵⁵ Results from multivariate analysis of positive PWC in women with endometrial cancer have been decidedly mixed (Table 5.3).⁵⁶⁻⁶¹ Some gynecologic oncologists maintain that patient management should not be determined on the basis of a positive PWC result alone.⁶² Others contend that positive PWC is an independent prognostic variable and that adjuvant treatment should be considered.^{46,63} A randomized clinical trial, with adjudicated cytologic diagnoses, might one day resolve this controversy.

Cervical Cancer

The results of peritoneal washing cytology are not included in the FIGO staging system for cervical cancer. The incidence of positive cytologic findings in patients who undergo primary definitive surgery for all stages of cervical cancer combined is relatively low: approximately 8%.⁶⁴ In patients with early-stage disease (FIGO stage IB) the incidence is even lower: approximately 1.2%.⁶⁴ For this reason, some investigators assert that PWC is unnecessary in patients with early-stage disease.⁶⁴ Others report a strong association between positive cytologic findings and other indicators of a poor prognosis, such as lymph node involvement, and contend that patients with positive cytology have a high recurrence rate in the peritoneal cavity.^{65,66} These investigators recommend using PWC as a guide for planning therapy for patients with advanced disease.

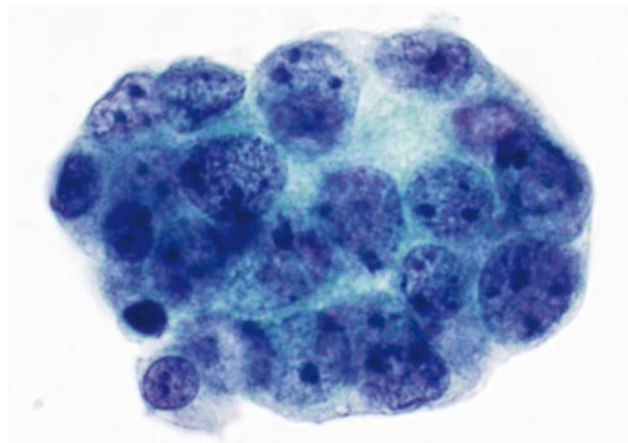


Figure 5.16 Endometrial carcinoma, endometrioid type. The cells are enlarged, crowded, and have coarsely textured chromatin. Because well-differentiated endometrial cancers have round nuclei and only mild atypia, they can be difficult to distinguish from reactive mesothelial cells. The absence of intercellular windows is a helpful feature (Papanicolaou stain).

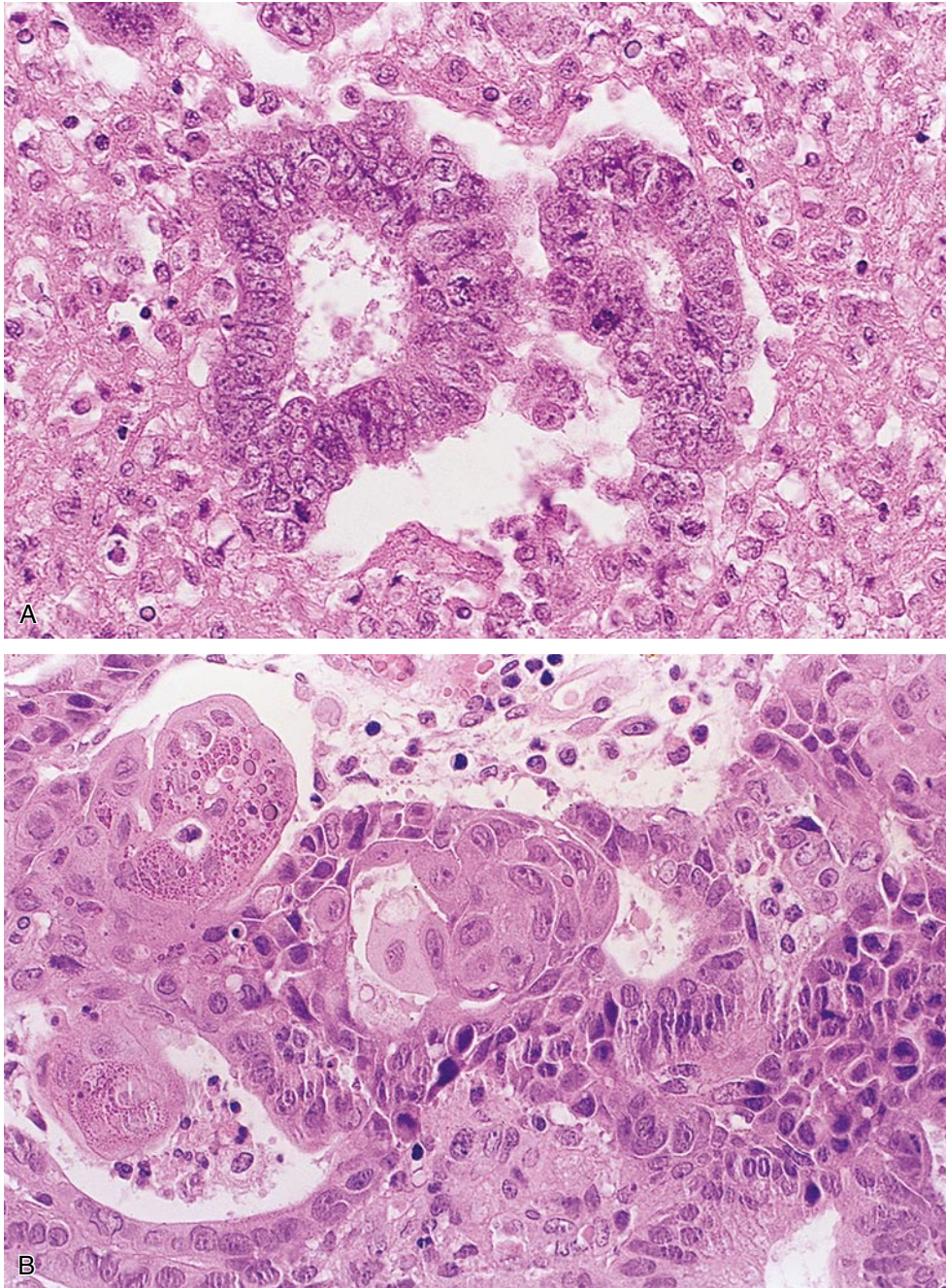


Figure 5.17 Endometrial carcinoma, endometrioid type. A, Cell block sections occasionally show well-formed neoplastic glands. A mitotic figure is present (hematoxylin-eosin [H & E] stain). B, Cell block sections may show squamous morule formation by the neoplastic cells (H & E stain).

Positive PWC results are more often found in patients with adenocarcinoma than with squamous cell carcinoma of the cervix.⁶⁵⁻⁶⁸ Adenocarcinoma of the cervix in peritoneal washings can take one of several patterns, ranging from rare isolated cells to abundant clusters and isolated cells with enlarged nuclei and prominent nucleoli.⁶⁸ Squamous cell carcinoma is easily recognized when the tumor cells have the characteristic cytoplasmic orangeophilia seen in keratin-

ized tumors. Nonkeratinized tumors are harder to identify, and mimic sheets of crowded mesothelial cells.⁶⁸ They have a tendency to exfoliate either as large clusters with smooth, rounded contours or as dense sheets with elongated, irregular outlines. Nuclei are enlarged, with coarsely textured chromatin, and nucleoli can be prominent. The abnormal nuclei and the high nuclear-to-cytoplasmic ratio helps distinguish these sheets from benign mesothelial cells.

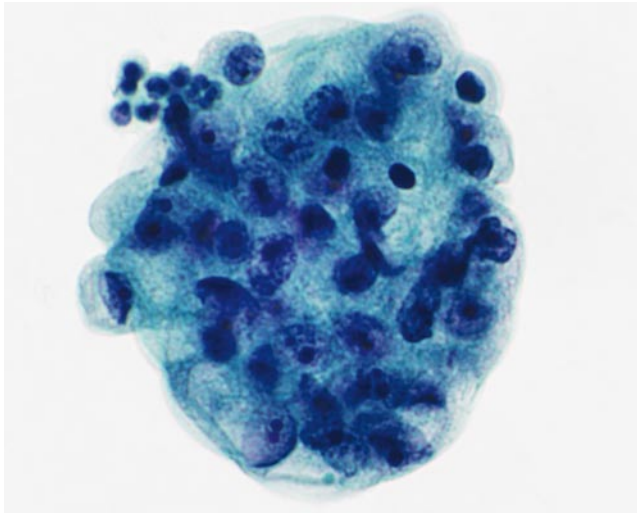


Figure 5.18 Endometrial carcinoma, clear cell type. High-grade endometrial carcinomas like the clear cell type have large nuclei and prominent nucleoli. The clear cell cancers have abundant clear cytoplasm (Papanicolaou stain).

Other Malignancies

Fallopian tube cancers resemble ovarian cancers in many ways; they have similar risk factors, share most of the same histologic tumor types, and have a similar biologic behavior. For these reasons, the staging system for fallopian tube cancers is similar to that for ovarian cancers.⁵ Positive peritoneal washings modify stage I and II tumors to stage Ic and IIc, respectively.

Peritoneal washing cytology is sometimes used to document peritoneal spread of gastric and pancreatic cancers. Positive results are common in patients with pancreatic (21% to 30%)⁶⁹⁻⁷¹ and gastric cancers (21% to 43%).^{20,70,72-75} A lower frequency has been reported for patients with colorectal cancer (3% to 15%),^{70,76} although some investigators have found higher rates.⁷⁷

Staging of pancreatic cancers aims to identify patients with resectable disease. Contrast-enhanced computed tomography (CT) is reliable when positive for metastases, but many patients with a negative CT scan are found to be

TABLE 5.3 PERITONEAL WASHING CYTOLOGY IN WOMEN WITH ENDOMETRIAL CANCER: ITS VALUE AS AN INDEPENDENT PROGNOSTIC VARIABLE

First Author, Year	Number of Endometrial Cancers	FIGO Stages Examined	Positive Cytology (%)	Independent Variable?
Konski et al., 1988 ^a	134	Clinical I	14	No
Grimshaw et al., 1990 ^b	322	Clinical I	5	No
Kadar et al., 1992 ^c	269	Clinical I, II	13	No
Ebina et al., 1997 ^d	114	Surgical I, II, III, IV	35	No
Takeshima et al., 2001 ^e	534	Non-FIGO subgroups	22	No
Mariani et al., 2002 ^f	51	Surgical IIIA	82	No
Fadare et al., 2005 ^g	220	Surgical I, II	8.6	No
Turner et al., 1989 ^h	567	Surgical I	4.9	Yes
Morrow et al., 1991 ⁱ	895	Clinical I, II	11	Yes
Descamps et al., 1997 ^j	144	Clinical I, II	9.7	Yes
Kashimura et al., 1997 ^k	199	Clinical I, II, III, IV	15	Yes
Obermair et al., 2001 ^l	369	Clinical I	3.5	Yes
Santala et al., 2003 ^m	43	Surgical II-IV	35	Yes

^aKonski A, Poulter C, Keys H, et al.: Absence of prognostic significance, peritoneal dissemination and treatment advantage in endometrial cancer patients with positive peritoneal cytology. *Int J Radiat Oncol Biol Phys* 1988;4:49-55.

^bGrimshaw RN, Tupper WC, Fraser RC, et al.: Prognostic value of peritoneal cytology in endometrial carcinoma. *Gynecol Oncol* 1990;36:97-100.

^cKadar N, Homesley HD, Malfetano JH: Positive peritoneal cytology is an adverse factor in endometrial carcinoma only if there is other evidence of extrauterine disease. *Gynecol Oncol* 1992;46:145-149.

^dEbina Y, Hareyama H, Sakuragah N, et al.: Peritoneal cytology and its prognostic value in endometrial carcinoma. *Int Surg* 1997;82(3):244-248.

^eTakeshima N, Nishida H, Tabata T, et al.: Positive peritoneal cytology in endometrial cancer: Enhancement of other prognostic indicators. *Gynecol Oncol* 2001;82(3):470-473.

^fMariani A, Webb MJ, Keeney GL, et al.: Assessment of prognostic factors in stage IIIA endometrial cancer. *Gynecol Oncol* 2002;86(1):38-44.

^gFadare O, Mariappan MR, Hileeto D, et al.: Upstaging based solely on positive peritoneal washing does not affect outcome in endometrial cancer. *Mod Pathol* 2005;18(5):673-680.

^hTurner DA, Gershenson DM, Atkinson N, et al.: The prognostic significance of peritoneal cytology for stage I endometrial cancer. *Obstet Gynecol* 1989;74:775-780.

ⁱMorris PC, Haugen J, Anderson B, Buller R: The significance of peritoneal cytology in Stage IB cervical cancer. *Obstet Gynecol* 1992;80:196-198.

^jDescamps P, Calais G, Moire C, et al.: Predictors of distant recurrence in clinical stage I or II endometrial carcinoma treated by combination surgical and radiation therapy. *Gynecol Oncol* 1997;64(1):54-58.

^kKashimura M, Sugihara K, Toki N, et al.: The significance of peritoneal cytology in uterine cervix and endometrial cancer. *Gynecol Oncol* 1997;67(3):285-290.

^lObermair A, Geramou M, Tripcony L, et al.: Peritoneal cytology: Impact on disease-free survival in clinical stage I endometrioid adenocarcinoma of the uterus. *Cancer Letters* 2001;164:105-110.

^mSantala M, Talvensaari-Mattila A, Kauppila A: Peritoneal cytology and preoperative serum CA 125 level are important prognostic indicators of overall survival in advanced endometrial cancer. *Anticancer Res* 2003;23(3C):3097-3103.

unresectable at surgical exploration. Laparoscopy allows identification of metastatic disease beyond the resolution of CT: 24% of patients with stage 1 or 2 localized pancreatic cancer have visible metastases by laparoscopy; another 10% have microscopic disease detected by PWC.⁶⁹

Similarly, laparoscopic lavage identifies patients with gastric cancer who are unlikely to be cured by resection. Between 4% and 16% of patients without visible metastases have positive PWC.²⁰ By multivariate analysis, PWC is an independent prognostic factor for survival in patients with gastric cancer^{72,75} and is included in the Japanese staging system for gastric cancer.⁷⁵

MONITORING RESPONSE TO TREATMENT ("SECOND-LOOK PROCEDURES")

Women with advanced ovarian cancer may undergo subsequent surgery to assess their response to primary cytoreductive surgery and adjuvant therapy. Such "second-look procedures" (followed by "third-look," and so on) are sometimes needed because radiographic studies and measurement of serum CA-125 levels are insufficiently accurate as measures of residual disease. After the incision is made, peritoneal washings are obtained, and the peritoneum is inspected and palpated. If no tumor is visible, random biopsies are taken from vari-

ous peritoneal locations, the omentum, and the retroperitoneal lymph nodes. More than 50% of women prove to have residual disease. Unfortunately, earlier initiation of treatment does not affect survival, and therefore second-look procedures are currently recommended only for women in research protocols.¹³

PWC in this setting is hampered by low sensitivity for malignancy; findings are negative in 31% to 86% of biopsy-proven residual disease^{9,11,21,22,24-26} most likely because adhesions cause poor distribution of the lavage fluid. In addition, chemotherapy and radiotherapy produce alterations in normal mesothelial cells that may cause them to be misinterpreted as malignant.

CYTOMORPHOLOGY OF TREATMENT EFFECT:

- enlarged, multinucleated mesothelial cells
- large nuclei
- normochromatic
- prominent nucleoli
- abundant cytoplasm

Normal mesothelial cells altered by chemotherapy or radiation show frequent multinucleation and marked variation in nuclear size (anisonucleosis; Fig. 5.19). Despite the often prominent nuclear enlargement, cytoplasm is usually abundant, and thus the normal nuclear-to-cytoplasmic ratio is unchanged. Chromatin is usually

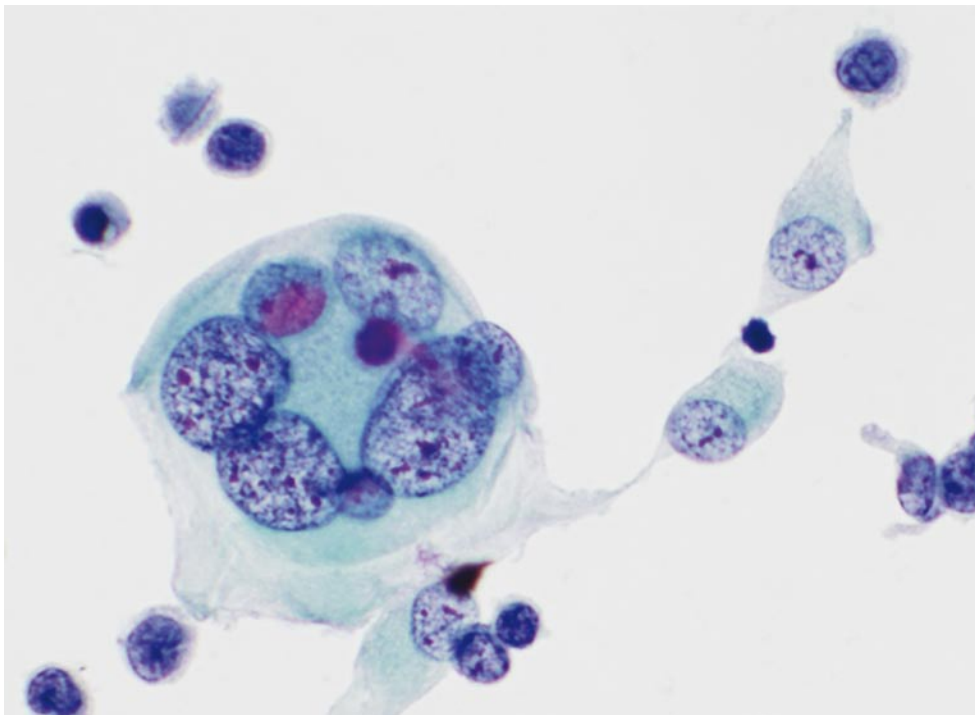


Figure 5.19 Mesothelial cell atypia as a result of chemotherapy. There is multinucleation and marked anisonucleosis, but only slight hyperchromasia. Cytoplasm is abundant (Papanicolaou stain).

pale and finely granular. Nucleoli may be prominent. In contrast, malignant cells often have a highly increased nuclear-to-cytoplasmic ratio and more coarsely textured chromatin.⁷⁸ In ambiguous cases it is often extremely helpful to compare the washings with histologic sections from the patient's known malignancy. The distinction is important, because the decision to continue therapy depends, in part, on the results of PWC.

REFERENCES

1. Keetel WC, Elkins HB: Experience with radioactive colloidal gold in the treatment of ovarian carcinoma. *Am J Obstet Gynecol* 1956;71:553-5687
2. Creasman WT, Rutledge F: The prognostic value of peritoneal cytology in gynecologic malignant disease. *Am J Obstet Gynecol* 1971;110:773-81.
3. Keetel WC, Pixley EE, Buchsbaum HJ: Experience with peritoneal cytology in the management of gynecologic malignancies. *Am J Obstet Gynecol* 1974;120:174-182.
4. FIGO Cancer Committee: Staging announcement. *Gynecol Oncol* 1986;25:383-385.
5. Heintz AP, Odicino F, Maisonneuve P, et al.: Carcinoma of the fallopian tube. FIGO 6th annual report on the results of treatment in gynecological Cancer. *Int J Gynaecol Obstet* 2006;95 Suppl 1:S145-S160.
6. FIGO Cancer Committee: Announcements. *Gynecol Oncol* 1989;35:125-127.
7. Mathew S, Erozan YS: Significance of peritoneal washings in gynecologic oncology: The experience with 901 intraoperative washings at an academic medical center. *Arch Pathol Lab Med* 1997;121:604-606.
8. Fadare O, Mariappan MR, Hileeto D, et al.: Upstaging based solely on positive peritoneal washing does not affect outcome in endometrial cancer. *Mod Pathol* 2005;18(5):673-680.
9. Copeland LJ, Gershenson DM, Wharton JT, et al.: Microscopic disease at second-look laparotomy in advanced ovarian cancer. *Cancer* 1985;55:472-478.
10. Patel NP, Taylor CA, Levine EA, et al.: Cytomorphologic features of primary peritoneal mesothelioma in effusion, washing, and fine-needle aspiration biopsy specimens: Examination of 49 cases at one institution, including post-intraperitoneal hyperthermic chemotherapy findings. *Am J Clin Pathol* 2007;128(3):414-422.
11. Kudo R, Takashina T, Ito E, et al.: Peritoneal washing cytology at second-look laparotomy in cisplatin-treated ovarian cancer patients. *Acta Cytol* 1990;34:545-548.
12. Heintz AP, Odicino F, Maisonneuve P, et al.: Carcinoma of the ovary. FIGO 6th annual report on the results of treatment in gynecological cancer. *Int J Gynaecol Obstet* 2006;95 Suppl 1:S161-S192.
13. Fader AN, Rose PG: Role of surgery in ovarian carcinoma. *J Clin Oncol* 2007;25(20):2873-2883.
14. Jacques SM, Selvaggi SM: Multiple peritoneal cytologies collected during laparotomy for gynecologic malignancy. *Diagn Cytopathol* 1991;7:482-486.
15. Ziselman EM, Harkavy SE, Hogan M, et al.: Peritoneal washing cytology: Uses and diagnostic criteria in gynecologic neoplasms. *Acta Cytol* 1984;28:105-110.
16. Selvaggi SM: Diagnostic pitfalls of peritoneal washing cytology and the role of cell blocks in their diagnosis. *Diagn Cytopathol* 2003;28(6):335-341.
17. Pretorius RG, Lee KR, Papillo J, et al.: False-negative peritoneal cytology in metastatic ovarian carcinoma. *Obstet Gynecol* 1986;68:619-623.
18. Sidawy MK, Silverberg SG: Endosalpingiosis in female peritoneal washings: A diagnostic pitfall. *Int J Gynecol Pathol* 1987;6:340-346.
19. McGowan L: Peritoneal cytology as an indicator of disease in patients with residual ovarian cancer [Letter]. *Obstet Gynecol* 1989;73:136-137.
20. Burke EC, Karpeh MS, Conlon KC, Brennan MF: Peritoneal lavage cytology in gastric cancer: An independent predictor of outcome. *Ann Surg Oncol* 1998;5:411-415.
21. Lowe E, McKenna H: Peritoneal washing cytology: A retrospective analysis of 175 gynecological patients. *Aust NZ Obstet Gynecol* 1989;29:55-61.
22. Ravinsky E: Cytology of peritoneal washings in gynecologic patients: Diagnostic criteria and pitfalls. *Acta Cytol* 1986;30:8-16.
23. Zuna RE, Mitchell ML: Cytologic findings in peritoneal washings associated with benign gynecologic disease. *Acta Cytol* 1988;32:139-147.
24. Coffin CM, Adcock LL, Dehner LP: The second-look operation for ovarian neoplasms: A study of 85 cases emphasizing cytologic and histologic problems. *Int J Gynecol Pathol* 1985;4:97-109.
25. Rubin SC, Dulane ED, Markman M, et al.: Peritoneal cytology as an indicator of disease in patients with residual ovarian carcinoma. *Obstet Gynecol* 1988;71:851-853.
26. Webb MJ, Snyder JA, Williams TJ, Decker DG: Second-look laparotomy in ovarian cancer. *Gynecol Oncol* 1982;14:285-293.
27. Covell JL, Carry JB, Feldman PS: Peritoneal washings in ovarian tumors: potential sources of error in cytologic diagnosis. *Acta Cytol* 1985;29:310-316.
28. Sneye N, Fernandez T, Copeland LJ, Katz RL: Müllerian inclusions in peritoneal washings: potential source of error in cytologic diagnosis. *Acta Cytol* 1986;30:271-276.
29. Zuna RE, Mitchell ML, Mulick KA, Weijchert WM: Cytohistologic correlation of peritoneal washing cytology in gynecologic disease. *Acta Cytol* 1989;33:327-336.
30. Harouny VR, Sutton GP, Clark SA, et al.: The importance of peritoneal cytology in endometrial carcinoma. *Obstet Gynecol* 1988;72:394-398.
31. Slade AJ, Dieterich M, Sturgis CD: Eosinophilic metaplastic atypia in exfoliated cells of ovarian endometriosis: A potential cytodifferential pitfall in peritoneal fluids. *Diagn Cytopathol* 2004;31(2):123-125.
32. Sams VR, Benjamin E, Wart RHT: Ectopic pancreas: A cause of false-positive peritoneal cytology. *Acta Cytol* 1990;34:641-644.
33. Shield P: Peritoneal washing cytology. *Cytopathology* 2004;15(3):131-141.
34. Wojcik EM, Naylor B: "Collagen balls" in peritoneal washings: prevalence, morphology, origin, and significance. *Acta Cytol* 1992;36:466-470.
35. Nascimento A, Hornstein MD, Crum CP: Benign conditions of the ovary. In Crum CP, Lee KR (eds): *Diagnostic Gynecologic and Obstetric Pathology*. Philadelphia, Elsevier 2006, p. 716.
36. Sidawy MK, Chandra P, Oertel XC: Detached ciliary tufts in female peritoneal washings: A common finding. *Acta Cytol* 1987;31:841-844.
37. Stowell SB, Wiley CM, Perez-Reyes N, Powers CN: Cytologic diagnosis of peritoneal fluids. Applicability to the laparoscopic diagnosis of endometriosis. *Acta Cytol* 1997;41(3):817-822.
38. Jemal A, Siegel R, Ward E, et al.: Cancer statistics, 2007. *CA Cancer J Clin* 2007;57(1):43-66.
39. Johnson TL, Kumar NB, Hopkins M, Hughes JD: Cytologic features of ovarian tumors of low malignant potential in peritoneal fluids. *Acta Cytol* 1988;32:513-518.
40. Guo M, Lim JC, Wojcik EM: Pelvic washing cytology of ovarian Sertoli-Leydig-cell tumor with retiform pattern: A case report. *Diagn Cytopathol* 2003;29(1):28-30.
41. International Federation of Gynecology and Obstetrics: Corpus cancer staging. *Int J Gynecol Obstet* 1989;28:190.

42. Turner DA, Gershenson DM, Atkinson N, et al.: The prognostic significance of peritoneal cytology for stage I endometrial cancer. *Obstet Gynecol* 1989;74:775-780.
43. Eltabbakh GH, Piver MS, Hempling RE, Shin KH: Excellent long-term survival and absence of vaginal recurrences in 332 patients with low-risk stage I endometrial adenocarcinoma treated with hysterectomy and vaginal brachytherapy without formal staging lymph-node sampling: Report of a prospective trial. *Int J Radiat Oncol Biol Phys* 1997;38:373-380.
44. Grimshaw RN, Tupper WC, Fraser RC, et al.: Prognostic value of peritoneal cytology in endometrial carcinoma. *Gynecol Oncol* 1990;36:97-100.
45. Konski A, Poulter C, Keys H, et al.: Absence of prognostic significance, peritoneal dissemination and treatment advantage in endometrial cancer patients with positive peritoneal cytology. *Int J Radiat Oncol Biol Phys* 1988;4:49-55.
46. Obermair A, Geramou M, Tripcony L, et al.: Peritoneal cytology: Impact on disease-free survival in clinical stage I endometrioid adenocarcinoma of the uterus. *Cancer Letters* 2001;164:105-110.
47. Mulvany NJ, Arnstein M, Ostor AG: Fallopian tube cytology: A histocorrelative study of 150 washings. *Diagn Cytopathol* 1997;16:483-488.
48. Obermair A, Geramou M, Gucer F, et al.: Does hysteroscopy facilitate tumor cell dissemination? Incidence of peritoneal cytology from patients with early stage endometrial carcinoma following dilatation and curettage (D & C) versus hysteroscopy and D & C. *Cancer* 2000;88:139-143.
49. Biewenga P, de Blok S, Birnie E: Does diagnostic hysteroscopy in patients with stage I endometrial carcinoma cause positive peritoneal washings? *Gynecol Oncol* 2004;93(1):194-198.
50. Sonoda Y, Zerbe M, Smith A, et al.: High incidence of positive peritoneal cytology in low-risk endometrial cancer treated by laparoscopically assisted vaginal hysterectomy. *Gynecol Oncol* 2001;80(3):378-382.
51. Eltabbakh GH, Mount SL: Laparoscopic surgery does not increase the positive peritoneal cytology among women with endometrial carcinoma. *Gynecol Oncol* 2006;100(2):361-364.
52. LoKW, CheungTH, YimSF, et al.: Prospective self-controlled study on prevention of hysteroscopic dissemination in endometrial carcinoma. *Int J Gynecol Cancer* 2004;14(5):921-926.
53. Geszler G, Szpak CA, Harris RE, et al.: Prognostic value of peritoneal washings in patients with malignant mixed müllerian tumors of the uterus. *Am J Obstet Gynecol* 1986;155:83-89.
54. Kanbour AI, Buchsbaum HJ, Hall A, Kanbour AI: Peritoneal cytology in malignant mixed Müllerian tumors of the uterus. *Gynecol Oncol* 1989;33:91-95.
55. Arikian G, Reich O, Weiss U, et al.: Are endometrial carcinoma cells disseminated at hysteroscopy functionally viable? *Gynecol Oncol* 2001;83(2):221-226.
56. Ebina Y, Hareyama H, Sakuraguchi N, et al.: Peritoneal cytology and its prognostic value in endometrial carcinoma. *Int Surg* 1997;82(3):244-248.
57. Takeshima N, Nishida H, Tabata T, et al.: Positive peritoneal cytology in endometrial cancer: Enhancement of other prognostic indicators. *Gynecol Oncol* 2001;82(3):470-473.
58. Mariani A, Webb MJ, Keeney GL, et al.: Assessment of prognostic factors in stage IIIA endometrial cancer. *Gynecol Oncol* 2002;86(1):38-44.
59. Descamps P, Calais G, Moire C, et al.: Predictors of distant recurrence in clinical stage I or II endometrial carcinoma treated by combination surgical and radiation therapy. *Gynecol Oncol* 1997;64(1):54-58.
60. Kashimura M, Sugihara K, Toki N, et al.: The significance of peritoneal cytology in uterine cervix and endometrial cancer. *Gynecol Oncol* 1997;67(3):285-290.
61. Santala M, Talvensaaari-Mattila A, Kauppila A: Peritoneal cytology and preoperative serum CA 125 level are important prognostic indicators of overall survival in advanced endometrial cancer. *Anticancer Res* 2003;23(3C):3097-3103.
62. Kadar N, Homesley HD, Malfetano JH: Positive peritoneal cytology is an adverse factor in endometrial carcinoma only if there is other evidence of extrauterine disease. *Gynecol Oncol* 1992;46:145-149.
63. Morrow C, Bundy BB, Kurman RJ, et al.: Relationship between surgical-pathological risk factors and outcome in clinical stage I and II carcinoma of the endometrium: A Gynecologic Oncology Group study. *Gynecol Oncol* 1991;40:55-65.
64. Morris PC, Haugen J, Anderson B, Buller R: The significance of peritoneal cytology in Stage IB cervical cancer. *Obstet Gynecol* 1992;80:196-198.
65. Imachi M, Tsukamoto N, Matsuyama T, Nakano H: Peritoneal cytology in patients with carcinoma of the uterine cervix. *Gynecol Oncol* 1987;26:202-207.
66. Ito K, Noda K: Peritoneal cytology in patients with uterine cervical carcinoma. *Gynecol Oncol* 1992;47:76-79.
67. Abu-Ghazaleh S, Johnston W, Creasman WT: The significance of peritoneal cytology in patients with carcinoma of the cervix. *Gynecol Oncol* 1984;17:139-148.
68. Zuna RE, Hansen K, Mann W: Peritoneal washing cytology in cervical carcinoma: Analysis of 109 patients. *Acta Cytol* 1990;34:645-651.
69. Jimenez RE, Warshaw AL, Fernandez-del Castillo C: Laparoscopy and peritoneal cytology in the staging of pancreatic cancer. *J Hepatobiliary Pancreat Surg* 2000;7:15-20.
70. Martin JK, Jr., Goellner JR: Abdominal fluid cytology in patients with gastrointestinal malignant lesions. *Mayo Clin Proc* 1986;61:467-471.
71. Yamada S, Takeda S, Fujii T, et al.: Clinical implications of peritoneal cytology in potentially resectable pancreatic cancer: Positive peritoneal cytology may not confer an adverse prognosis. *Ann Surg* 2007;246(2):254-258.
72. Bando E, Yonemura Y, Takeshita Y, et al.: Intraoperative lavage for cytological examination in 1,297 patients with gastric carcinoma. *Am J Surg* 1999;178:256-262.
73. To EM, Chan WY, Chow C, et al.: Gastric cancer cell detection in peritoneal washing: Cytology versus RT-PCR for CEA transcripts. *Diagn Mol Pathol* 2003;12(2):88-95.
74. Yajima K, Kanda T, Ohashi M, et al.: Clinical and diagnostic significance of preoperative computed tomography findings of ascites in patients with advanced gastric cancer. *Am J Surg* 2006;192(2):185-190.
75. Kodera Y, Yamamura Y, Shimizu Y, et al.: Peritoneal washing cytology: Prognostic value of positive findings in patients with gastric carcinoma undergoing a potentially curative resection. *J Surg Oncol* 1999;72(2):60-64; discussion 64-65.
76. Gozalan U, Yasti AC, Yuksek YN, et al.: Peritoneal cytology in colorectal cancer: Incidence and prognostic value. *Am J Surg* 2007;193(6):672-675.
77. Kanellos I, Demetriades H, Zintzaras E, et al.: Incidence and prognostic value of positive peritoneal cytology in colorectal cancer. *Dis Colon Rectum* 2003;46(4):535-539.
78. Sagae S, Berek JS, Fu YS, et al.: Peritoneal cytology of ovarian cancer patients receiving intraperitoneal therapy: Quantification of malignant cells and response. *Obstet Gynecol* 1988;72:782-788.

Cerebrospinal Fluid

EDMUND S. CIBAS

ANATOMY AND PHYSIOLOGY

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REPORTING TERMINOLOGY

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Carcinoma of the Breast

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Atypical Teratoid/Rhabdoid Tumor

Choroid Plexus Tumors

Pineal Tumors

Germ Cell Tumors

Other Tumors of the Central Nervous System

The lumbar puncture (spinal tap) was introduced in 1891,^{1,2} and in 1904 a French neurologist first described malignant cells in cerebrospinal fluid (CSF).³ Since then, preparatory methods have been refined and diagnostic features described in a number of monographs and atlases,⁴⁻⁷ attesting to the importance of CSF cytology for excluding leptomeningeal metastasis in a patient with neurologic symptoms.

CSF circulates in the *subarachnoid space*, formed by the *leptomeninges* (composed of the pia mater and arachnoid mater), over cerebral and spinal surfaces. Fluid is then reabsorbed by the arachnoid granulations into the venous system and the cycle begins anew.^{8,9}

The total volume of CSF in the adult is about 150 mL. Approximately 500 mL/day is produced (0.35 mL/min), meaning that the CSF is renewed three or four times daily.

ANATOMY AND PHYSIOLOGY

The brain contains four cavities known as *ventricles*, which communicate with each other and with the subarachnoid space surrounding the brain and spinal cord. The ventricles are lined by a cuboidal ciliated cell layer called the *ependyma*. In some areas the ependyma differentiates into a complex villous structure called the *choroid plexus*, which is composed of a single layer of cuboidal nonciliated cells overlying a vascular core. The choroid plexus produces most of the CSF by both filtering plasma across capillary walls and actively secreting fluid. After leaving the ventricles via the midline foramen of Magendie and the two lateral foramina of Luschka,

OBTAINING AND PREPARING THE SPECIMEN

Most CSF specimens are obtained by lumbar puncture (LP), in which a needle is passed through the intervertebral space at L3 to L4 or L4 to L5. Rarely, because of inflammation at these sites or a bony abnormality, the specimen must be obtained from the cisterna magna at the base of the brain. Cisternal CSF sometimes yields positive results when LP is negative.¹⁰ CSF is sometimes aspirated directly from a lateral ventricle during a neurosurgical procedure; such specimens often contain fragments of normal brain. In patients undergoing chemotherapy for leptomeningeal metastases, a silicone pouch (Ommaya reservoir) is

implanted in subcutaneous tissue, and a cannula leads from the pouch into a lateral ventricle through a 3-mm burr hole (Fig. 6.1). This is an efficient way to introduce chemotherapeutic drugs and to withdraw CSF periodically for examination.

A minimum of 1 mL should be collected for cytologic evaluation, but 3 mL or more is preferable,¹¹ and at least 10 mL is considered ideal.¹² Surprisingly, the amount of CSF removed does not affect the likelihood that a patient will develop a headache.^{13,14}

Fluid should be collected fresh and delivered to the laboratory as quickly as possible to prevent cellular deterioration. If the specimen cannot be prepared immediately, it should be refrigerated at 4°C. If a delay of more than 48 hours is anticipated, the sample can be preserved by adding an equal volume of 50% ethanol. A CSF sample for cytologic examination should never be frozen.

The most common ways to prepare specimens are by cytocentrifugation, membrane filtration, and thinlayer preparation. Because greater technical skill is needed to process membrane filtration specimens, this is best done in a laboratory that specializes in this method. Cytocentrifugation has greater flexibility because both alcohol-fixed and air-dried slides can be prepared using this method. Lymphoid cells are best evaluated using air-dried preparations, thus it is advisable to prepare both alcohol-fixed Papanicolaou-stained slides and air-dried Wright-stained slides. Depending on the volume and cellularity of the specimen, additional slides can be used for immunocytochemical studies if needed.

Blood is a common contaminant. The needle lacerates the veins that course along the crowded nerve roots of the cauda equina in 75% of LPs,¹⁵ and this can create diagnostic problems. Neutrophils, if accompanied by red blood cells, should not necessarily be interpreted as evidence of acute meningitis. Similarly, leukemic blasts from peripheral blood can contaminate the fluid, leading to an erroneous impression of meningeal involvement by leukemia. It takes only a minute amount of blood to alter the cellular composition of CSF significantly—an amount that can be invisible to the naked eye.¹⁶ Because alcohol fixation lyses red blood cells, contamination is best detected on air-dried preparations. If red blood cells are present, the possibility of contamination by peripheral blood blasts should be raised.

REPORTING TERMINOLOGY

As with most cytologic specimens, CSF cytology results are commonly reported as either “negative for malignancy,” “atypical,” “suspicious,” or “positive.” Over 90% of CSF specimens are assigned a cytologic diagnosis of “negative for malignant cells.”¹⁷ Many show only a small number of lymphocytes and monocytes, essentially a normal CSF.

The primary role of CSF cytology is to exclude circulating malignant cells in CSF pathways. Although a specific diagnosis of some benign diseases (e.g., cryptococcosis) can be made cytologically, in most nonmalignant central

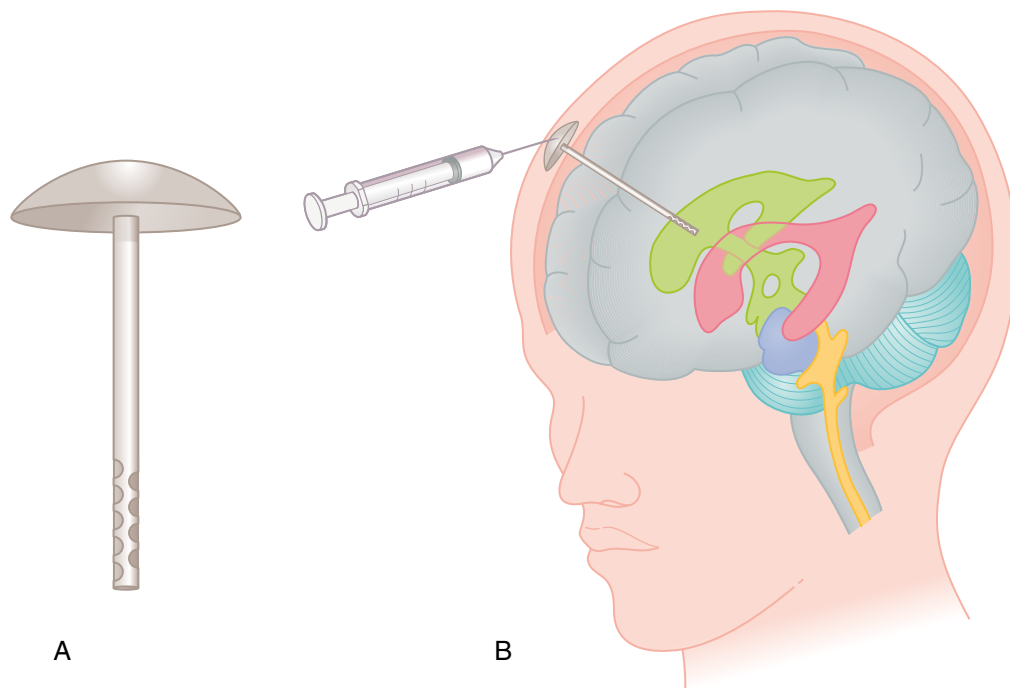


Figure 6.1 Ommaya reservoir. A, The Ommaya reservoir consists of a pouch connected to a cannula with distal perforations. B, When implanted, the pouch is subcutaneous and the cannula ends in one of the lateral ventricles. A needle penetrates the pouch to instill drugs and withdraw cerebrospinal fluid (CSF).

nervous system (CNS) diseases CSF cytology is frustratingly unrevealing. The myriad diseases that cause aseptic meningitis, for example, have as their final common denominator nothing more than a lymphocytic or monocytic pleocytosis. The cause is identified by other clinical and laboratory methods.

ACCURACY

The sensitivity of CSF cytology for detecting malignant cells is about 60%.¹⁸⁻²⁰ But sensitivity depends on several factors. First and foremost, a positive CSF sample will occur only if a CNS malignancy actually invades into a ventricle or the subarachnoid space (Fig. 6.2).

The sensitivity of cerebrospinal fluid cytology depends on:

- the number of specimens examined
- the volume of fluid submitted
- the extent of leptomeningeal disease
- the site from which the sample was obtained (lumbar versus cisterna magna)

The sensitivity of a single cytologic examination is 54% but increases to 84% with a second sample.²⁰ Smaller incremental increases in sensitivity are observed with more than two specimens.²¹ Sensitivity is dependent on sample volume; sensitivity is greater if 10 mL are submitted rather than 3 mL.¹² Sensitivity also depends on the extent of leptomeningeal disease: 38% for focal and 66% for disseminated leptomeningeal tumor.¹⁸ Similarly, only 50% of patients with early meningeal involvement by acute lymphoblastic leukemia have a suspicious or positive CSF.²² The site from which the sample is obtained

can also affect the sensitivity; if LP specimens are negative, a tap from the cisterna magna sometimes yields malignant cells.¹⁰

The specificity of CSF cytology is high. False-positive diagnoses are estimated at 2% to 3%.²³ The most common cause is the overdiagnosis of lymphoma or leukemia, particularly in patients with herpes zoster meningitis,¹⁸ cryptococcal meningitis,^{24,25} Lyme disease,²⁶ viral meningitis,²⁴ and in LP specimens contaminated with blood.²⁴

In light of these data, the American College of Physicians has determined that cytologic examination of CSF for meningeal malignancy has moderate sensitivity and high specificity.²⁷

NORMAL ELEMENTS

Normal CSF is sparsely cellular and, in adults, contains less than 5 cells/mm³ (equivalent to 5000 cells/mL). In newborns the fluid is more cellular.²⁸

Benign cells in CSF:

- common
 - lymphocytes
 - monocytes
- rare
 - choroid plexus or ependymal cells
 - brain fragments
 - germinal matrix
 - chondrocytes
 - bone marrow

Normal CSF is composed of lymphocytes and monocytes (Fig. 6.3). Only small numbers of mature lymphocytes are present in normal CSF, but their number can

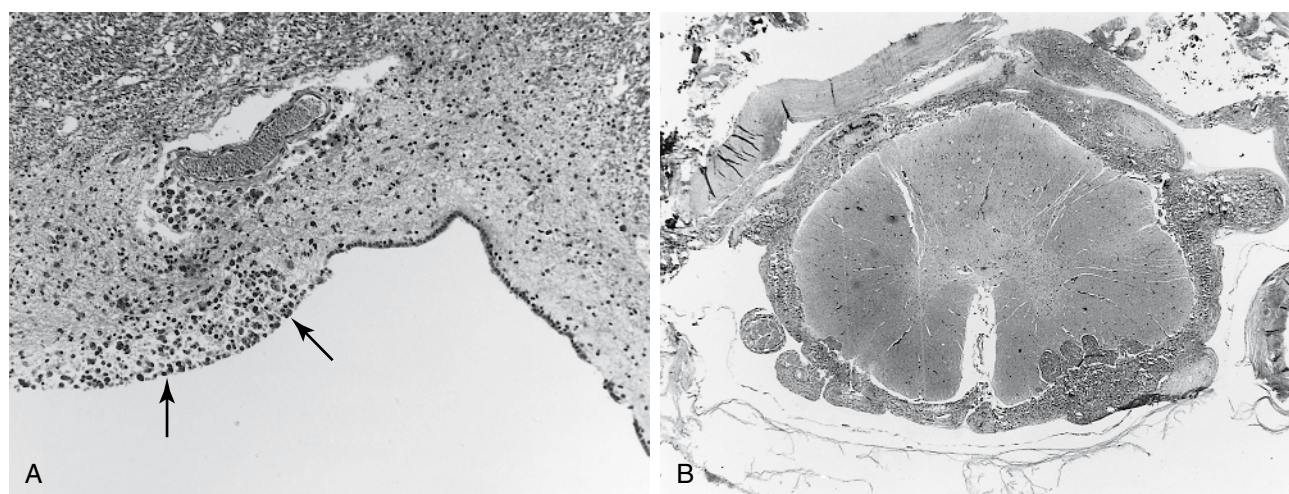


Figure 6.2 Tumor involvement of cerebrospinal fluid (CSF) pathways. *A*, The arrows indicate a focus of astrocytoma cells that have disrupted the ependymal lining. *B*, The normally delicate subarachnoid membrane is filled and expanded by metastatic breast cancer cells, encasing the spinal cord. CSF was positive for malignant cells in both cases.

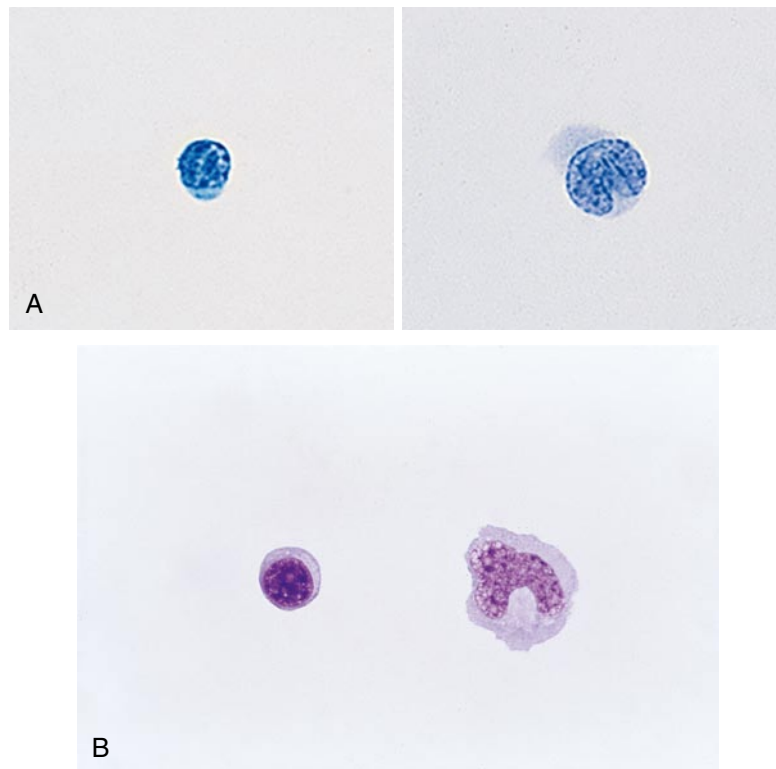


Figure 6.3 Normal cerebrospinal fluid (CSF). Lymphocytes and monocytes are the usual components of a normal CSF. A, Papanicolaou-stained cytocentrifuge preparation. B, Wright-Giemsa stain.

increase markedly, particularly in viral meningitis and other inflammatory or infectious conditions. Such specimens can be highly cellular, with a minority population of so-called “atypical” lymphoid cells that are large and may show irregular nuclear contours, with clefting, blebs, and nucleoli.

Monocytes are also present in normal CSF. Larger than lymphocytes, they have folded, kidney bean-shaped nuclei and a moderate amount of cytoplasm.

Choroid plexus and ependymal cells are seen in less than 0.5% of LP specimens.²⁹ These cells have round to oval, regular nuclei, a moderate amount of cytoplasm, and are seen singly or in small clusters (Fig. 6.4). Brain fragments have a fibrillary texture and contain glial cells, neurons, and capillaries (Fig. 6.5). They are seen in samples taken directly from the ventricles because the needle traverses brain parenchyma, and are not seen in LP samples. Rarely, isolated neurons are present (Fig. 6.6).

In CSF from neonates, most commonly those born prematurely, one occasionally sees small, immature cells of germinal matrix origin.^{30,31} Germinal matrix cells lie beneath the ependyma in the wall of the lateral ventricles and exfoliate when there is subependymal and intraventricular hemorrhage (Fig. 6.7). They are often clustered and molded to one another, thus mimicking a small cell malignancy. Because of their frequent association with hemorrhage, these cells are often accompanied by hemosiderin-laden macrophages.

If the LP needle is inserted too far anteriorly, the CSF can be contaminated by chondrocytes (Fig. 6.8) or bone marrow cells (Fig. 6.9) from the intervertebral disc or vertebral body, respectively. These cells, seen in less than 1% of CSF specimens,^{32,33} should not be mistaken for malignant cells.³⁴ Other common contaminants are starch granules and squamous cells. Although mitoses are more common in malignant specimens, they are occasionally seen in benign conditions such as bacterial and viral meningitis.³⁵

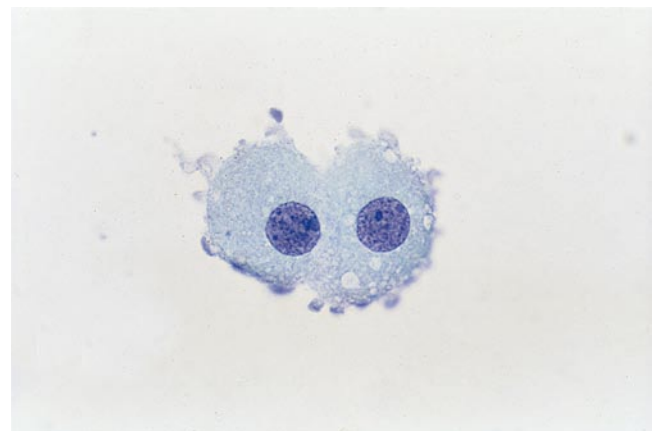


Figure 6.4 Choroid plexus or ependymal cells. These cells are rare in cerebrospinal fluid (CSF); even when present, their number is usually small. They may be isolated or arranged in small clusters. Note the dispersed chromatin texture of the nuclei and the abundant cytoplasm (Papanicolaou stain).

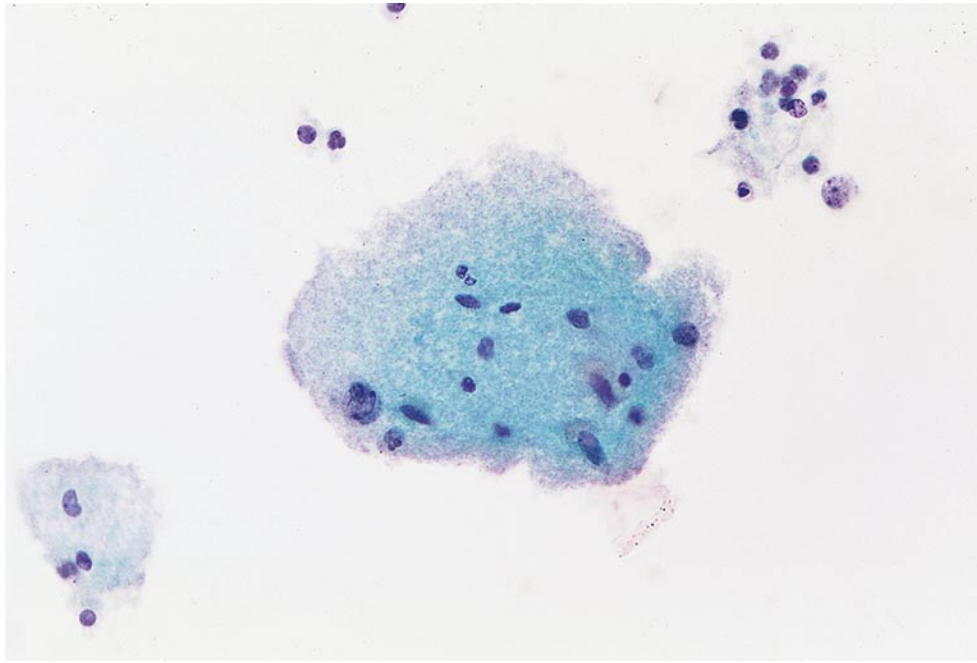


Figure 6.5 Brain tissue. This fragment, obtained from a ventricular tap, has a fibrillary texture and contains normal glial cells. Some fragments may contain neurons and capillaries (Papanicolaou stain).

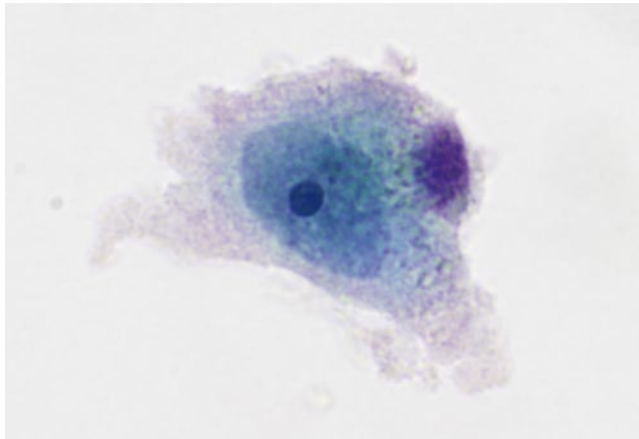


Figure 6.6 Neuron. Many neurons have an angular shape, a round nucleus, and a prominent nucleolus. Some have lipofuscin pigment (Papanicolaou stain).

ABNORMAL INFLAMMATORY CELLS

Inflammatory cells such as macrophages, plasma cells, and eosinophils are an abnormal finding in CSF. They may accompany malignancy, but are also seen in a variety of non-neoplastic conditions.

Macrophages have abundant, vacuolated cytoplasm that sometimes contains ingested cells, organisms, or pigment (Fig. 6.10).

Macrophages in cerebrospinal fluid are associated with:

- meningitis
- subarachnoid hemorrhage
- intraventricular hemorrhage
- cerebral infarction
- post-treatment inflammation
- multiple sclerosis³⁶

Plasma cells are also an abnormal but nonspecific finding in CSF (Fig. 6.11).

Plasma cells in cerebrospinal fluid are associated with:

- viral meningitis (e.g., enterovirus, human immunodeficiency virus [HIV])
- Lyme disease
- tuberculosis
- cysticercosis
- syphilis
- multiple sclerosis³⁶

Polymorphonuclear leukocytes are a normal finding if there is contamination by peripheral blood, but numerous neutrophils unaccompanied by a proportionate increase in red blood cells raise the possibility of acute meningitis (Fig. 6.12). In a patient with acquired

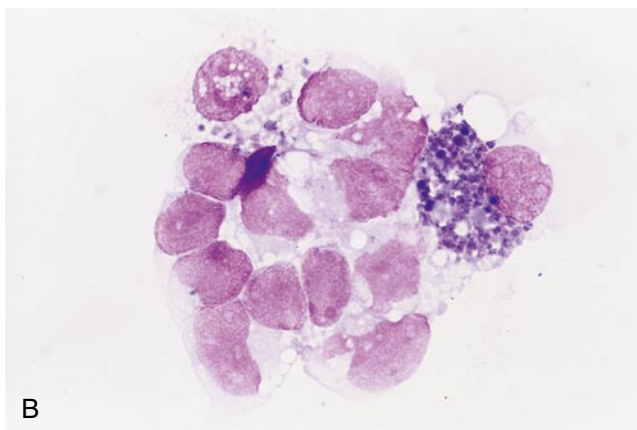
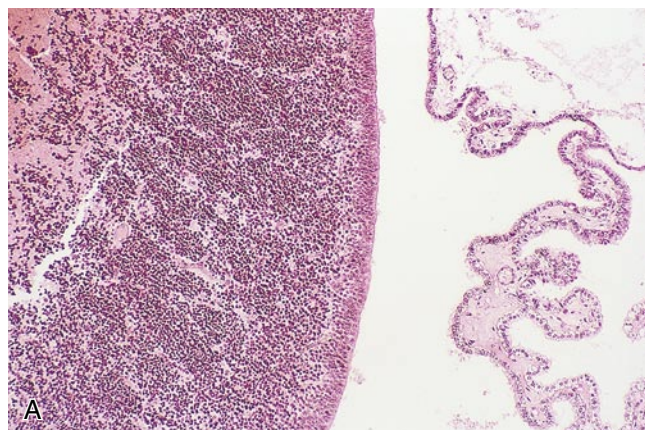


Figure 6.7 Germinal matrix. A, In neonates, the small, dark, densely packed cells of the germinal matrix lie beneath the ependyma, Choroid plexus is seen on the right (hematoxylin-eosin [H & E] stain). B, When the ependyma is injured, clusters of small cells, accompanied by macrophages, are seen in cerebrospinal fluid [CSF]; Wright-Giemsa stain).

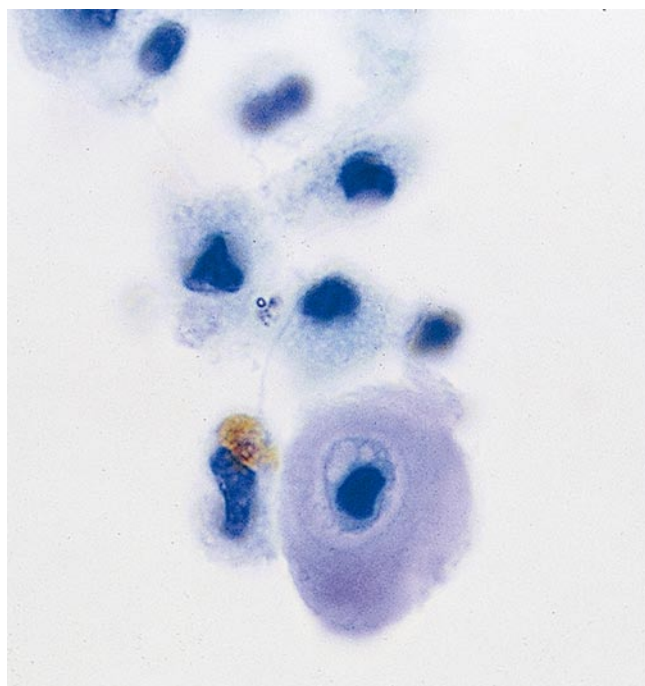


Figure 6.8 Chondrocyte. Rarely seen in cerebrospinal fluid (CSF), chondrocytes are large cells with pyknotic nuclei, surrounded by an extracellular mucopolysaccharide matrix that stains purple with the Papanicolaou stain (Papanicolaou stain).

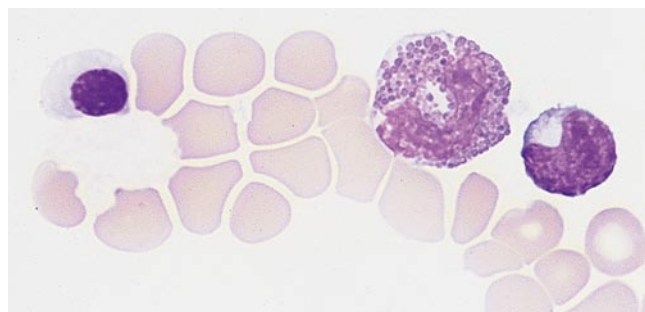


Figure 6.9 Bone marrow. If the needle penetrates a vertebral body, immature erythroid and myeloid elements from normal bone marrow are sampled, as seen here (Wright-Giemsa stain).

immune deficiency syndrome (AIDS), numerous neutrophils are highly suggestive of cytomegalovirus (CMV) radiculopathy. Viral cytopathic inclusions, however, are not seen. The diagnosis of CMV radiculopathy can be confirmed by viral culture.³⁷

DIFFERENTIAL DIAGNOSIS OF NEUTROPHILS IN CEREBROSPINAL FLUID:

- peripheral blood contamination
- acute bacterial meningitis
- CMV radiculopathy
- Toxoplasma meningoencephalitis
- viral meningitis (early stage)

Eosinophils are rare in CSF; when present, especially in large numbers (Fig. 6.13), they suggest a parasitic infection, particularly *Taenia solium* and *Angiostrongylus cantonensis*.³⁸

DIFFERENTIAL DIAGNOSIS OF EOSINOPHILS IN CEREBROSPINAL FLUID:

- parasites
- *Coccidioides immitis*
- ventriculoperitoneal shunts
- Rocky Mountain spotted fever

NON-NEOPLASTIC DISORDERS

Acute Bacterial Meningitis

Many bacteria can cause meningitis, including *Neisseria meningitidis* (meningococcus), *Hemophilus influenzae*, *Streptococcus pneumoniae* (pneumococcus; see Fig. 6.12), and *Listeria monocytogenes*.

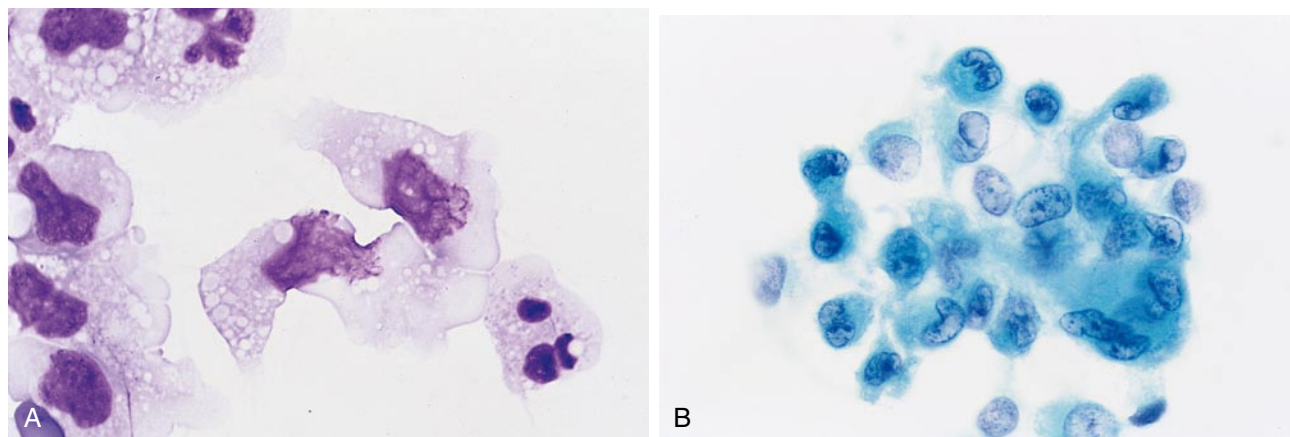


Figure 6.10 Macrophages. Macrophages have abundant cytoplasm and are seen in benign and malignant conditions. *A*, Subarachnoid hemorrhage (Wright-Giemsa stain). *B*, Chronic shunt infections (Papanicolaou stain).

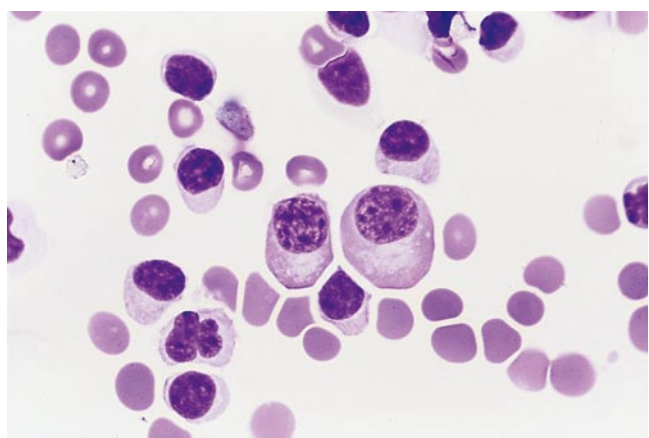


Figure 6.11 Plasma cells (Lyme disease). Cerebrospinal fluid (CSF) from patients with aseptic meningitis of any cause, as in this case of Lyme disease, can be markedly hypercellular, with plasma cells as a prominent component. Such specimens resemble a lymphoproliferative disorder (Wright-Giemsa stain).

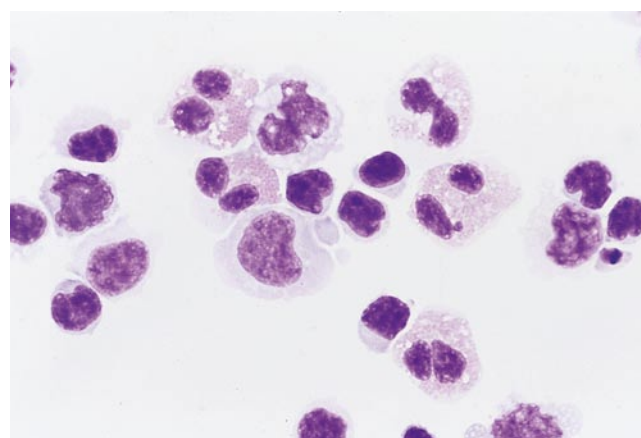


Figure 6.13 Eosinophils. Eosinophils are an abnormal finding in cerebrospinal fluid (CSF). When prominent, a parasitic infection should be considered (air-dried Wright-Giemsa stain).

CYTOMORPHOLOGY OF ACUTE BACTERIAL MENINGITIS:

- numerous neutrophils
- bacteria (may or may not be seen)

Because bacterial meningitis can be fatal if not treated immediately, prompt diagnosis is crucial. Any CSF sample composed predominantly of neutrophils should be considered high probability for bacterial meningitis; precise identification of the organism depends on microbiologic cultures.

DIFFERENTIAL DIAGNOSIS OF ACUTE BACTERIAL MENINGITIS:

- traumatic tap
- toxoplasma meningoencephalitis
- CMV radiculopathy
- aseptic meningitis (early)

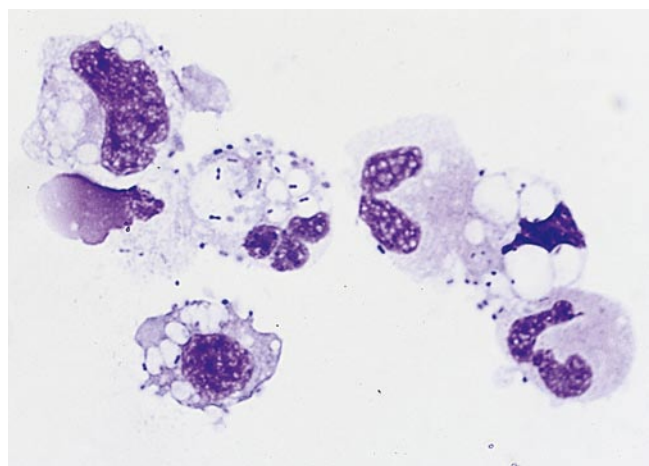


Figure 6.12 Acute bacterial (pneumococcal) meningitis. There are numerous neutrophils and bacteria (Wright-Giemsa stain).

Neutrophils admixed with red blood cells and other blood elements are a normal finding resulting from a traumatic tap. Abundant neutrophils are seen in other conditions, such as toxoplasmosis, CMV radiculopathy,³⁷ and in the early stages of aseptic meningitis.

Aseptic Meningitis

Aseptic meningitis is a misnomer, but the term is ingrained in clinical practice. Despite its name, it is most commonly caused by an infectious organism, usually a virus. The clinical course is less fulminant than that of acute bacterial (pyogenic) meningitis. Aseptic meningitis is usually self-limited and is treated symptomatically. The most common pathogen is one of the enteroviruses (non-paralytic poliovirus, echovirus, and coxsackieviruses).

CYTOMORPHOLOGY OF ASEPTIC MENINGITIS:

- increase in lymphocytes and monocytes
- small proportion of atypical lymphocytes

The cytologic findings are nonspecific. There is an increased number of predominantly small, mature lymphocytes, but also monocytes, plasma cells, and enlarged (so-called atypical) lymphocytes, some of which have prominent nucleoli and irregular nuclear contours. In the early stages, neutrophils are present (Fig. 6.14). Viral inclusions are rarely seen in CSF.³⁹

Aseptic meningitis is caused by a wide range of organisms, systemic diseases, and miscellaneous conditions (Table 6.1), with identical cytologic findings. It is seen in about 10% of patients within 1 to 2 weeks of seroconversion resulting from human immunodeficiency virus-1 (HIV-1). Aseptic meningitis occurs in some patients with Lyme disease.

A rare form of aseptic meningitis, *idiopathic recurrent meningitis*, also known as *Mollaret meningitis* (MM) after

the man who first described the disease in 1944, is characterized by recurring attacks of fever, headache, and neck stiffness. Symptoms appear suddenly, last for 5 to 7 days, resolve spontaneously, but recur days or years later. Herpes simplex viruses 1 and 2 have been identified as putative causative agents in some cases.^{40,41} The diagnosis of MM is made clinically after excluding other causes of aseptic meningitis. Cytologic findings are nonspecific, but there is often a marked predominance of monocytes.⁴² So-called “Mollaret cells,” monocytes with deep nuclear clefts that impart a footprint-like appearance to the nucleus, are seen within the first 24 hours of the onset of symptoms.⁴² They are characteristic of but not specific for MM; they can be seen in other diseases like sarcoidosis and Behçet disease.

DIFFERENTIAL DIAGNOSIS OF ASEPTIC MENINGITIS:

- primary CNS lymphoma
- secondary involvement by lymphoma
- acute leukemia

A polymorphous lymphoid population supports the diagnosis of a benign, reactive process. Nevertheless, the presence of some atypical lymphocytes raises the possibility of malignant lymphoma. Some lymphomas in the CNS are accompanied by numerous small, reactive T lymphocytes and can, in fact, mimic aseptic meningitis. Because the cells in most cases of aseptic meningitis are T cells,⁴³ immunocytochemistry and flow cytometry are useful in selected cases (Fig. 6.15). If all the lymphoid cells are T cells, a malignant lymphoma is unlikely, because most lymphomas, including primary CNS lymphomas, are B-cell neoplasms.⁴⁴

In contrast to viral meningitis, the meningeal infiltrate in Lyme disease is comprised of B cells (see Fig. 6.11). To distinguish the florid pleocytosis in some cases of Lyme disease from lymphoma, demonstration of polyclonal vs monoclonal expression of κ and λ light chains is necessary.²⁶

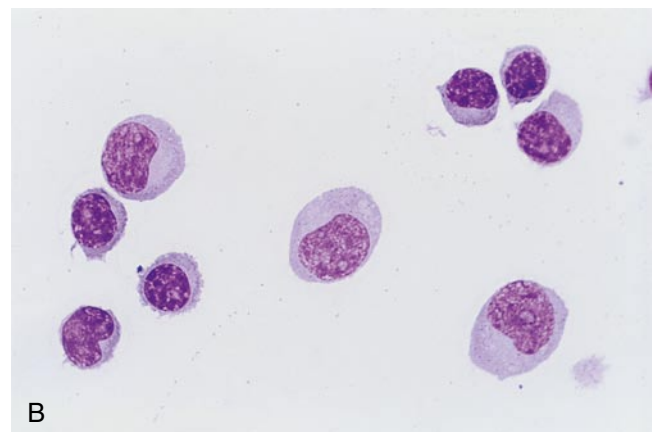
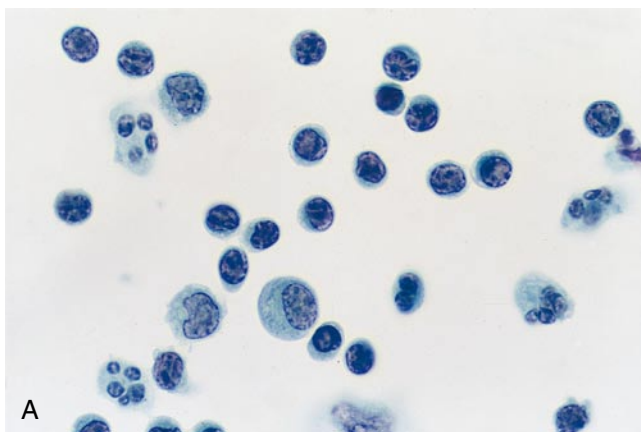


Figure 6.14 Aseptic meningitis (viral). An increased number of lymphocytes is present, including occasional so-called atypical lymphoid cells: larger cells with larger nuclei, some of which have irregular contours, more finely dispersed chromatin, and prominent nucleoli. Note that the predominant cell type is still the small mature lymphocyte. A, Papanicolaou stain. B, Wright-Giemsa stain.

TABLE 6.1 PARTIAL LIST OF CAUSES OF ASEPTIC MENINGITIS**Viruses**

- enteroviruses
- human immunodeficiency virus (HIV)
- herpes simplex virus (Mollaret meningitis)
- varicella–zoster virus
- arboviruses
- arenavirus (lymphocytic choriomeningitis)

Bacteria

- *Borrelia burgdorferi* (Lyme disease)
- *Treponema pallidum* (syphilis)
- *Mycobacterium tuberculosis*
- ehrlichiosis
- *Mycoplasma pneumoniae*

Fungi

- *Cryptococcus neoformans*
- *Histoplasma capsulatum*
- *Coccidioides immitis*

Systemic Diseases

- Behçet disease
- sarcoidosis

Other

- drugs
- vaccines
- parainfectious syndrome (acute disseminated encephalomyelitis)
- vasculitis

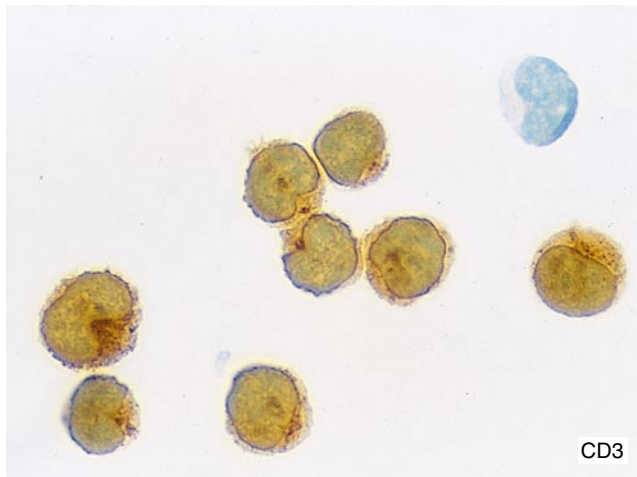


Figure 6.15 Aseptic meningitis. In most cases of aseptic meningitis, the lymphoid infiltrate is mostly T cells (immunocytochemistry for CD3, cytocentrifuge preparation).

Cryptococcal Meningitis

The only organism that is identified cytologically with any frequency in CSF is *Cryptococcus neoformans*, which causes disease in people who are both healthy and immunocompromised.

CYTOMORPHOLOGY OF CRYPTOCOCCAL MENINGITIS:

- round yeast forms
- variable size: 5 to 15 μm diameter
- pink or purple (Papanicolaou stain)
- asymmetric, narrow-based budding
- mucin-positive capsule
- refractile artifact

The degree of inflammation is variable.⁴⁵ There can be a marked lymphocytic or monocytic pleocytosis, in

which case organisms are hard to identify. Alternatively, there may be abundant organisms and little inflammatory response (Fig. 6.16). *C. neoformans* is sometimes perfectly round, but often indented. The indentations trap air under the coverslip, resulting in a crystal-like refractile artifact.

Toxoplasmosis

In patients who are immunocompromised, the protozoon *Toxoplasma gondii* can cause a variety of diseases of the CNS, including meningoencephalitis.

CYTOMORPHOLOGY OF TOXOPLASMA MENINGOENCEPHALITIS:

- neutrophils
- mononuclear cells
- tachyzoites

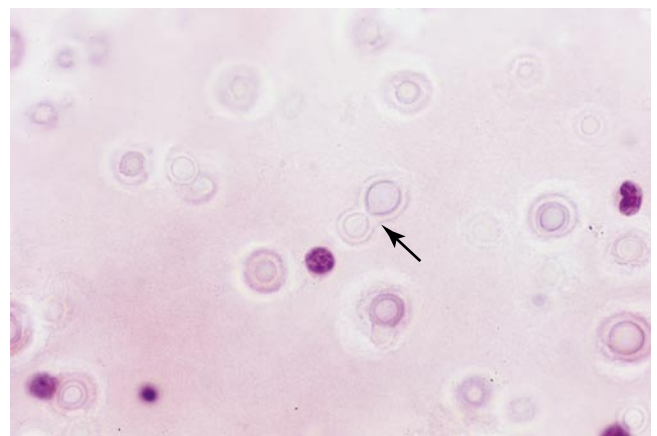


Figure 6.16 Cryptococcal meningitis. The organisms have a mucopolysaccharide capsule. Note the characteristic thin-necked budding (arrow; Papanicolaou stain).

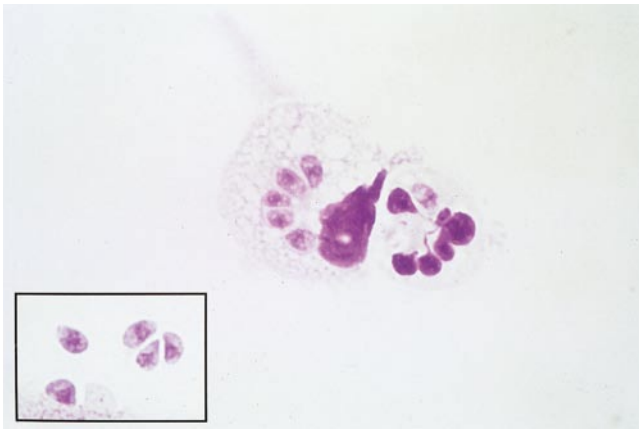


Figure 6.17 *Toxoplasma meningoencephalitis*. *Toxoplasmosis gondii* is a small bow-shaped organism with a single tiny nucleus. It can be intracellular or extracellular (inset) (Wright-Giemsa stain).

Toxoplasma tachyzoites are small, crescent-shaped organisms, 3 to 6 μm in length, with a tiny, round nucleus (Fig. 6.17). In patients who develop obstructive hydrocephalus, tachyzoites are more likely to be found in ventricular rather than lumbar samples.⁴⁶

Cysticercosis

Cerebral cysticercosis results from colonization of the brain by larvae of the tapeworm *Taenia solium*. Clinical symptoms are nonspecific. Imaging studies usually show a focal lesion in the brain.

CYTOMORPHOLOGY OF CYSTICERCOSIS:

- eosinophils (20% to 70% of cells)
- mononuclear cells

Larvae are not seen in the CSF. Serologic tests confirm the diagnosis.

Angiostrongyliasis

The lungworm *Angiostrongylus cantonensis* is endemic to Asia, particularly the Pacific region, but is found elsewhere. Infection, with subsequent migration of larvae to the CNS, results in an eosinophilic meningitis. Headache is the most common presenting symptom. The percentage of eosinophils in CSF is usually very high (20% to 70%). Larvae are occasionally seen in the fluid.⁴⁷ Focal lesions on computed tomography (CT) examination are usually absent, thus helping to distinguish angiostrongyliasis from cysticercosis. The disease is usually self-limited, and patients recover completely.

Other roundworm infections that usually present as eosinophilic meningitis are *Gnathostoma spinigerum*, a parasite of dogs and cats, and *Baylisascaris procyonis*, a parasite of racoons.⁴⁷

Primary Amebic Meningoencephalitis

The free living ameba *Naegleria fowleri* can cause acute meningoencephalitis. The organisms enter the subarachnoid space through the nose, often while a person is swimming in stagnant water or in an unchlorinated swimming pool. The organisms are best seen on wet preparations, where their motility can be appreciated.⁴⁸ With conventional cytologic preparations they can be difficult to distinguish from mononuclear cells. They have a relatively large nucleus and little cytoplasm, unlike *Entamoeba histolytica*. Fortunately, the disease is rare; it can be fatal within several days.

Primary amebic meningoencephalitis must be distinguished from an amebic brain abscess caused by *Entamoeba histolytica*. Amebae are not seen in the CSF with the latter infection.

NEOPLASMS

Involvement of the subarachnoid space by malignancy occurs in 5% to 8% of patients with cancer.⁴⁹ Some tumors, like small cell carcinoma of the lung (11%) and melanoma (20%), have a greater predilection than most for involving the leptomeninges.⁵⁰ Malignant cells gain access to the subarachnoid space by hematogenous dissemination, direct extension from a parenchymal brain lesion, or by tracking along spinal or cranial nerves. Involvement of the leptomeninges by tumor is often referred to as carcinomatous (or lymphomatous, etc.) meningitis, but the term *leptomeningeal metastasis* (LM) is best because it applies to all tumors that involved the meninges.

The clinical presentation of patients with LM is highly variable. Symptoms can include headache, mental changes, gait difficulty, diplopia, back pain, and lower extremity weakness. Gadolinium-enhanced MRI often shows suspicious enhancement of the leptomeninges when malignancy is present,⁵¹ but CSF for cytology is needed to confirm the diagnosis.

CSF examination is an important component in the diagnosis of LM. Many patients have elevated opening pressure, with elevated protein and depressed glucose levels.⁴⁹ Cytologic examination of CSF, however, is essential for documenting LM. When present, malignant cells are usually easily identified in CSF samples because they are strikingly different from the normal cells of CSF. Thus, most CSF samples are easily classified as either negative or positive for malignancy. When findings are inconclusive, often because of scant cellularity or poor preservation, a *suspicious* or *atypical* interpretation is warranted. Not surprisingly, the greatest difficulty is in the diagnosis of lymphoma and leukemia.

When malignant cells are identified, the clinical history often points to their site of origin. In most cases the

patient is known to have a malignancy, and the findings simply confirm metastasis. The most commonly encountered malignancies in CSF are lung and breast cancer, melanoma, lymphoma, and leukemia.

In about 10% of patients with a positive CSF cytologic examination provides the first documentation of a neoplasm.⁵² The lung is by far the most common occult primary site,⁵³⁻⁵⁵ followed by gastric cancer and melanoma. Patients with lymphoma, leukemia, or a primary CNS tumor can also present with a positive CSF examination. Curiously, a primary breast cancer is rarely occult when CSF involvement is detected.

Likely primaries in a patient with positive cerebrospinal fluid and no history of cancer:

- lung
- stomach
- melanoma
- lymphoma
- CNS

Metastatic tumors are much more frequent than primary CNS tumors in CSF; primary CNS tumors account for only 6% of all positive CSF samples.⁵²

Immunocytochemistry is not needed to diagnose most cases of LM.⁵⁶ In selected cases, however, it is useful for establishing a likely primary site, for example, in a patient whose cancer first manifests itself as a positive CSF. Epithelial markers can establish a diagnosis of carcinoma and exclude the possibility of lymphoma, melanoma, and a primary glioma. Glial fibrillary acidic protein (GFAP) differentiates glial from nonglial tissue and can be used to confirm a primary CNS origin for malignant cells in CSF.⁵⁷ GFAP is useful in establishing a diagnosis of malignancy when the cytologic examination is equivocal⁵⁸ because normal CSF obtained by LP does not contain GFAP-positive cells.⁵⁹

The median survival of untreated patients with LM is 1 month.⁴⁹ Treatment stabilizes or improves about 75% of patients, but it is almost always palliative rather than curative because current treatments cannot eliminate tumor from the subarachnoid space. The median survival of treated patients ranges from 4 to 10 months and depends in part on the tumor type: breast cancer and lymphoma respond better than most other tumors.⁴⁹ Radiation therapy is often considered in patients with bulky or symptomatic LM and is especially helpful in relieving pain and other symptoms. Intrathecal chemotherapy, administered by Ommaya reservoir, has been the mainstay of treatment for LM, but its palliative effects are usually short-lived.⁴⁹ The most commonly used intrathecal agents are methotrexate, cytarabine, and thiopeta. Systemic chemotherapy is gaining acceptance for treating LM because of its greater ability to penetrate bulkier tumor deposits.⁴⁹

Metastatic Solid Tumors

Carcinoma of the Lung

All four of the common histologic subtypes of lung cancer (adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and small cell carcinoma) can metastasize to the CSF pathways. Adenocarcinomas of the lung are common and squamous cell carcinomas uncommon in CSF.

CYTOMORPHOLOGY OF ADENOCARCINOMA OF THE LUNG:

- isolated cells or small clusters
- large cells
- abundant cytoplasm
- eccentric nucleus
- [Figure 6.18](#)

The differential diagnosis of metastatic adenocarcinoma of the lung to CSF includes macrophages, plasma cells, and ependymal or choroid plexus cells. Macrophages have smaller nuclei that are pale and often folded or curved, with granular and microvacuolated cytoplasm (see [Fig. 6.10](#)). Plasma cells are smaller than the cells of an adenocarcinoma of the lung, with more condensed chromatin and a tell-tale perinuclear hof. Ependymal or choroid plexus cells are rare in CSF; when present, they are few in number, usually with round, centrally placed nuclei.

CYTOMORPHOLOGY OF SMALL CELL CARCINOMA OF THE LUNG:

- isolated cells or clustered
- small cells
- nuclear molding
- karyorrhexis
- mitoses

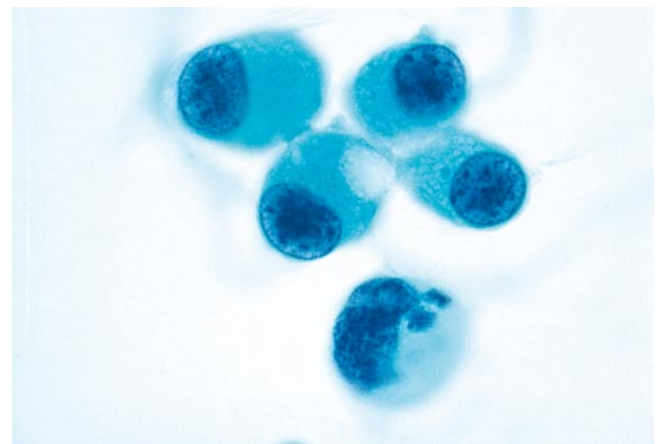


Figure 6.18 Adenocarcinoma of the lung. The malignant cells have large hyperchromatic nuclei and abundant cytoplasm. Note the eccentric placement of the nuclei (Papanicolaou stain).

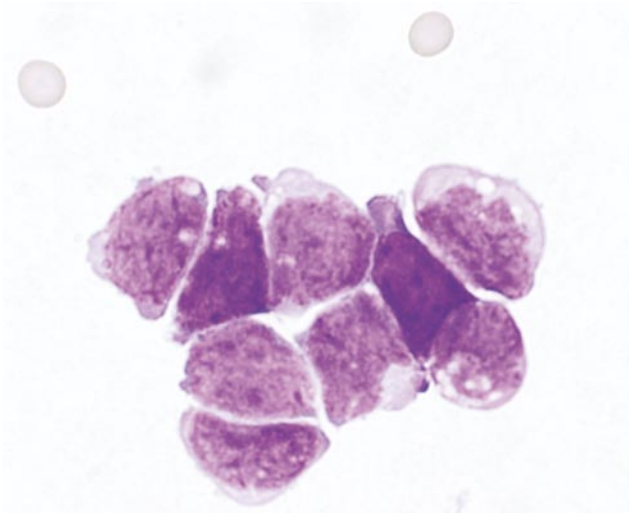


Figure 6.19 Small cell carcinoma of the lung. The small cells have dispersed chromatin, indistinct nucleoli, and scant cytoplasm. Nuclear molding is prominent (Wright-Giemsa stain).

Small cell carcinomas of the lung appear as small isolated or clustered cells in CSF. When isolated, they are easily mistaken for lymphocytes. Finding clusters with their characteristic molding is often essential for diagnosis (Fig. 6.19). Linear, molded arrangements with a “vertebral body” appearance are seen. The differential diagnosis includes other small cell malignancies, most of them pediatric, like medulloblastoma. A cytomorphic distinction is impossible, but this rarely presents a problem because patients have a history of small cell carcinoma or a suspicious lung mass.

Carcinoma of the Breast

Breast cancer not uncommonly metastasizes to the CSF. In contrast to lung cancer, it is rare that a positive CSF is the presenting finding in a woman with breast cancer.

CYTOMORPHOLOGY OF DUCTAL BREAST CANCER:

- isolated cells or small groups
- linear rows, rings (rare)
- large cells
- round nucleus
- prominent nucleolus
- scant cytoplasm (often)
- Figure 6.20

The large cannonball-like arrangements typical of breast cancer cells in pleural fluid (see Fig. 4.19A) are almost never seen in CSF. Instead, the malignant cells are usually dispersed as isolated cells. Nuclei are round or irregular, and some cells can be binucleated.

CYTOMORPHOLOGY OF LOBULAR BREAST CANCER:

- small-sized to medium-sized cells
- isolated cells
- signet ring shapes
- Figure 6.21

The differential diagnosis of ductal and lobular breast cancers is similar to that for adenocarcinoma of the lung and includes macrophages and ependymal/choroid plexus cells. Macrophages have smaller, pale, often folded or curved nuclei, with abundant granular and microvacuolated cytoplasm (see Fig. 6.10). Ependymal or choroid plexus cells are rare in CSF; when present, they are few in number and usually have a round, centrally placed nucleus (see Fig. 6.4). Some lobular breast cancer cells in CSF form linear arrangements reminiscent of the vertebral body-like structures seen in patients with small cell carcinoma.

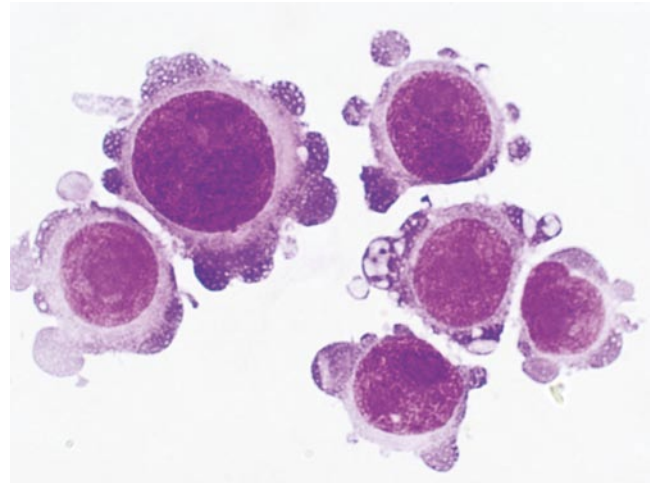


Figure 6.20 Ductal carcinoma of the breast. The malignant cells of this Bloom-Richardson grade 3 ductal cancer are round, large, and highly variable in size. Cytoplasmic blebs are commonly seen (Wright-Giemsa stain).

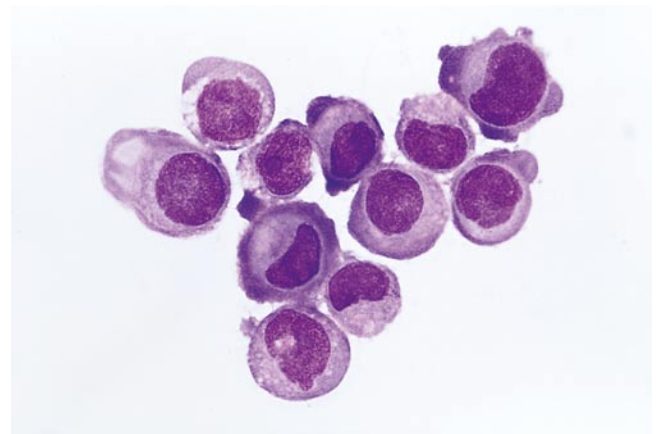


Figure 6.21 Lobular carcinoma of the breast. These malignant cells have round or semilunar nuclei, and some have prominent nucleoli (Wright-Giemsa stain).

Melanoma

Melanoma can metastasize to the meninges from a primary in the skin or other site, but it can also (rarely) arise in the leptomeninges as a primary CNS neoplasm. In a condition known as melanosis cerebri, the meninges contain melanocytes; presumably, it is these cells that give rise to leptomeningeal melanomas.^{60,61}

CYTOMORPHOLOGY OF MELANOMA:

- large cells
- macronucleolus
- melanin (not always)
- melanophages
- Figure 6.22

With a history of melanoma, the diagnosis is straightforward in most cases. Rarely, as in a patient without a documented melanoma whose malignant cells lack visible melanin, the distinction from a carcinoma may require immunocytochemistry. Stains for HMB45 and S-100 are generally positive, whereas stains for keratin and epithelial membrane antigen are negative.

Leukemia

Acute Lymphoblastic Leukemia

Acute lymphoblastic leukemia (ALL), a malignancy of lymphocyte precursors that arises from the bone marrow, is the most common malignancy in children. The peak incidence is between the ages of 2 and 7 years, but adults are also affected. Patients often present with pallor and weakness because of anemia, and bleeding resulting from thrombocytopenia. They can have lymphadenopathy, hepatosplenomegaly, and an anterior mediastinal mass. The diagnosis is made by bone marrow aspiration and

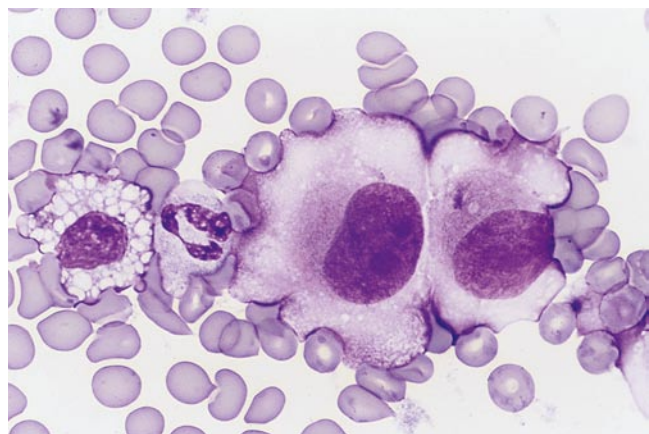


Figure 6.22 Melanoma. Melanoma cells tend to be isolated or only loosely aggregated. The two cells shown here are amelanotic (Wright-Giemsa stain).

biopsy, which shows replacement of normal elements by lymphoblasts. CNS involvement is present at initial diagnosis in 5% of cases and is usually occult.⁶² In fact, 75% of patients with ALL and LM are asymptomatic.⁶³

The appearance of the blasts is variable. According to the French-American-British (FAB) classification system, ALL is divided into types L1, L2, and L3 based on the cytomorphologic appearance on air-dried Wright-Giemsa stained preparations (Fig. 6.23A-C).⁶⁴ The World Health Organization (WHO) recommends that the FAB classification be abandoned because morphological classification has no clinical or prognostic relevance. Instead, the WHO advocates the use of a combined immunophenotypic and cytogenetic classification that carries prognostic significance. Nevertheless, knowledge of the spectrum of morphologic appearances of ALL can be quite useful in evaluating CSF, particularly in correlating CSF findings with prior bone marrow aspirates.

CYTOMORPHOLOGY OF ACUTE LYMPHOBLASTIC LEUKEMIA (USING WRIGHT-GIEMSA TYPE STAINS):

- L1
 - small blasts
 - round nucleus (rare clefts)
 - fine chromatin
 - inconspicuous nucleolus
 - scant, slightly basophilic cytoplasm
- L2
 - larger blasts
 - irregular nucleus
 - fine chromatin
 - prominent nucleolus
 - abundant cytoplasm
- L3
 - coarse chromatin
 - multiple nucleoli
 - dark blue cytoplasm
 - small cytoplasmic vacuoles (lipid)
 - indistinguishable from Burkitt lymphoma

In a small percentage of cases, the cells of ALL contain azurophilic cytoplasmic inclusions that resemble myeloid granules.

The treatment of ALL in children is one of the great successes of chemotherapy. At one time ALL was almost invariably fatal; today nearly 60% of children are long-term survivors of the disease. CNS prophylaxis is a mainstay of therapy. Without CNS prophylaxis, more than 50% of patients would develop CNS leukemia. Although prophylactic treatment of the CNS with radiotherapy to the cranium and intrathecal methotrexate has reduced the incidence of such relapses dramatically, they still occur in 5% to 10% of cases. Therefore, periodic monitoring of CSF for the presence of blasts is essential.

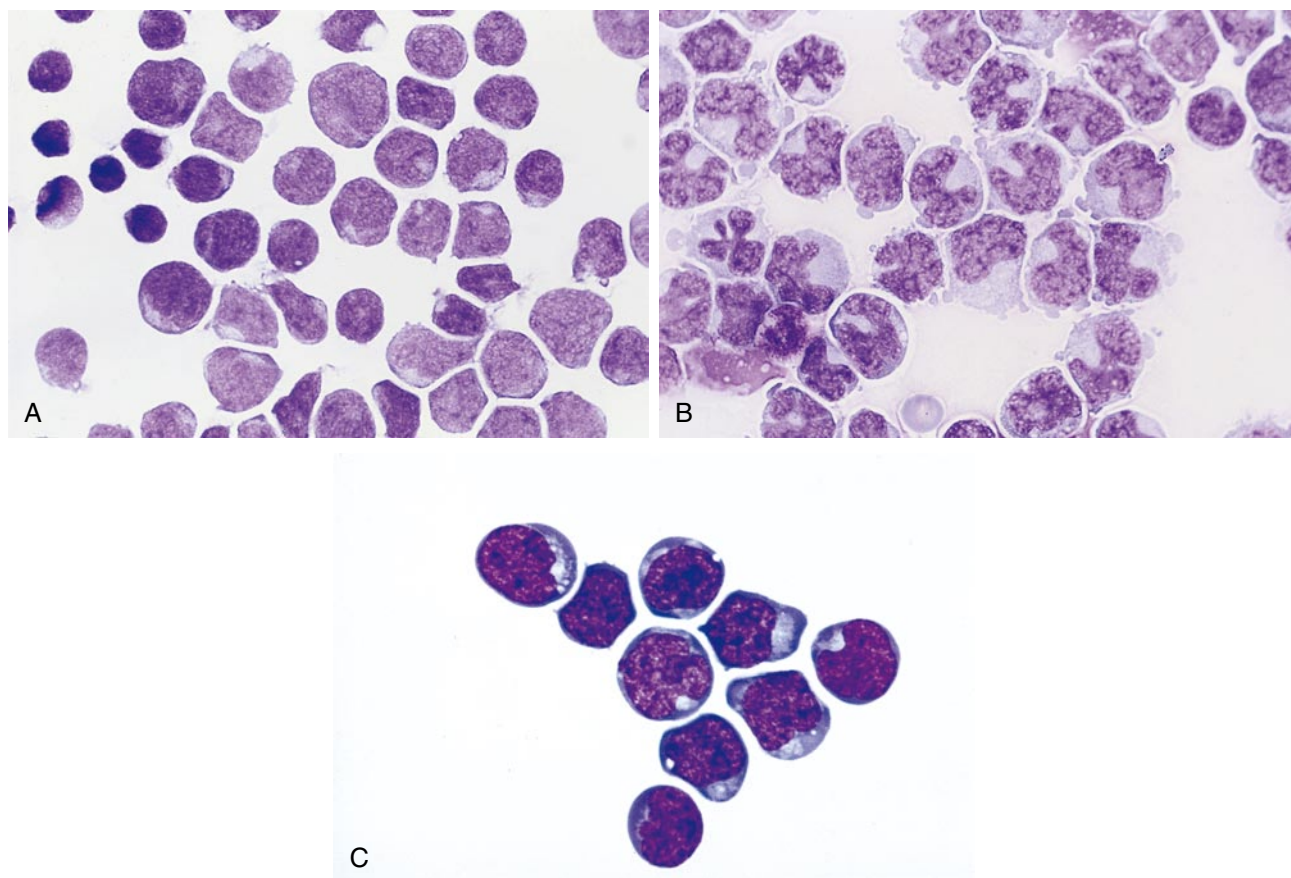


Figure 6.23 Acute lymphoblastic leukemia (ALL). Depending on the subtype, the blasts of ALL may have several different appearances, which are best appreciated on air-dried preparations. A, L1 blasts are small, with predominantly round nuclei and scant cytoplasm. B, L2 blasts are larger, with irregular nuclei and more abundant cytoplasm. C, L3 blasts are indistinguishable from the cells of Burkitt lymphoma. (A-C, Wright-Giemsa stain).

DIFFERENTIAL DIAGNOSIS OF ACUTE LYMPHOBLASTIC LEUKEMIA:

- reactive lymphocytes (e.g., aseptic meningitis)
- peripheral blood contamination

When the sample is hypercellular, the diagnosis is usually straightforward, particularly with air-dried Wright-Giemsa stained slides, which accentuate the characteristic morphologic features. Sparsely cellular samples can be difficult. Immunocytochemistry for terminal deoxynucleotidyl transferase (Tdt), a nuclear enzyme found in 90% to 95% of L1 and L2 leukemias (but not in type L3), can be extremely useful in distinguishing blasts from reactive lymphocytes, which are consistently negative for this marker. An aliquot of fluid is also often sent to the hematology laboratory, and blasts, if present, are noted in the differential count. Contamination of CSF by blasts in peripheral blood must be excluded¹⁶ because this can result in a false-positive diagnosis.²⁴ If CSF contains leukemic blasts admixed with red blood cells, the specimen is essentially noncontributory. The only exception is a patient in remission, whose peripheral blood counts are negative for blasts. In such a patient, a traumatic tap with blasts is diagnostic of a CSF recurrence.

Acute Myeloid Leukemia

The acute myeloid leukemias (AML) are neoplastic proliferations of the myeloid progenitor cells: immature granulocytes, monocytes, erythrocytes, and megakaryocytes. They are more common in adults than in children. AML can involve CSF either at presentation or subsequently,⁶⁵ although CSF involvement is less common than in ALL.

The FAB system recognizes eight morphologic types of AML based on bone marrow aspirates⁶⁶:

- AML undifferentiated (M0)
- AML without maturation (M1)
- AML with maturation (M2)
- acute promyelocytic leukemia (M3)
- acute myelomonocytic leukemia (M4)
- acute monocytic leukemia (M5)
- erythroleukemia (M6)
- acute megakaryoblastic leukemia (M7)

These subtypes have somewhat different clinical presentations. For example, it is more common for patients with acute myelomonocytic leukemia (AML M4) to have CNS involvement at the time of presentation than for those with other types of AML.⁶⁵ As with ALL, the WHO recommends abandoning the FAB classification for AML and

replacing it with a system that relies more on clinical and cytogenetic criteria.^{67,68} The WHO subtypes of AML are:

- AML with recurrent cytogenetic abnormalities
- AML with multilineage dysplasia
- AML and myelodysplastic syndrome, therapy related
- AML, not otherwise categorized

CYTOMORPHOLOGY OF ACUTE MYELOID LEUKEMIAS:

- round or highly irregular nucleus
- fine chromatin
- prominent nucleolus
- azurophilic granules (sometimes)
- mature granulocytes (some subtypes)

As with ALL, characteristic features of AML are best seen on Wright-Giemsa stained preparations (Fig. 6.24). Although azurophilic granules can be present, they are not diagnostic of AML because they are also seen in some cases of ALL. An important finding in many but not all cases of AML is the Auer rod. This linear structure is a crystallization of azurophilic granules and is pathognomonic of a myeloid disorder. More mature cells such as granulocytes can be present. In evaluating the CSF, it is usually sufficient simply to identify blasts because definitive diagnosis of ALL or AML and their subtypes depend on the evaluation of the bone marrow aspirate and biopsy.

Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) predominantly affects adults and has a long and protracted clinical

course. Despite this, LM is extremely uncommon.^{63,69,70} The cells of CLL are morphologically indistinguishable from small, mature lymphocytes.⁷¹ It is important, therefore, to exclude peripheral blood contamination from a traumatic tap; if the sample contains a significant number of red blood cells and the patient is known to have circulating leukemic cells at the time of specimen procurement, the CSF sample is essentially noncontributory. Even if red blood cells are scant or absent, immunophenotyping of the lymphoid cells must be performed⁷² to exclude a meningitis as a result of a compromised immune system.

Chronic Myeloproliferative Diseases

The chronic myeloproliferative diseases (CMPDs) are clonal hematopoietic stem cell disorders characterized by a bone marrow proliferation of granulocytic, erythroid, or megakaryocytic cells.⁶⁸ Clinically heterogeneous, they include chronic myelogenous leukemia, chronic neutrophilic leukemia, chronic eosinophilic leukemia (and the hypereosinophilic syndrome), polycythemia vera, chronic idiopathic myelofibrosis (with extramedullary hematopoiesis), essential thrombocythemia, and chronic myeloproliferative disease, unclassifiable. During the chronic phase, the neoplastic cells are minimally invasive, confined to bone marrow, blood, liver, and spleen, and they virtually never involve the CSF. Unfortunately, most patients eventually develop “blast crisis,” a transformation to acute leukemia that is almost always fatal, involving the CNS in some patients.⁷³ As with other leukemias, opportunistic infections are common and must be suspected when a specimen is examined.

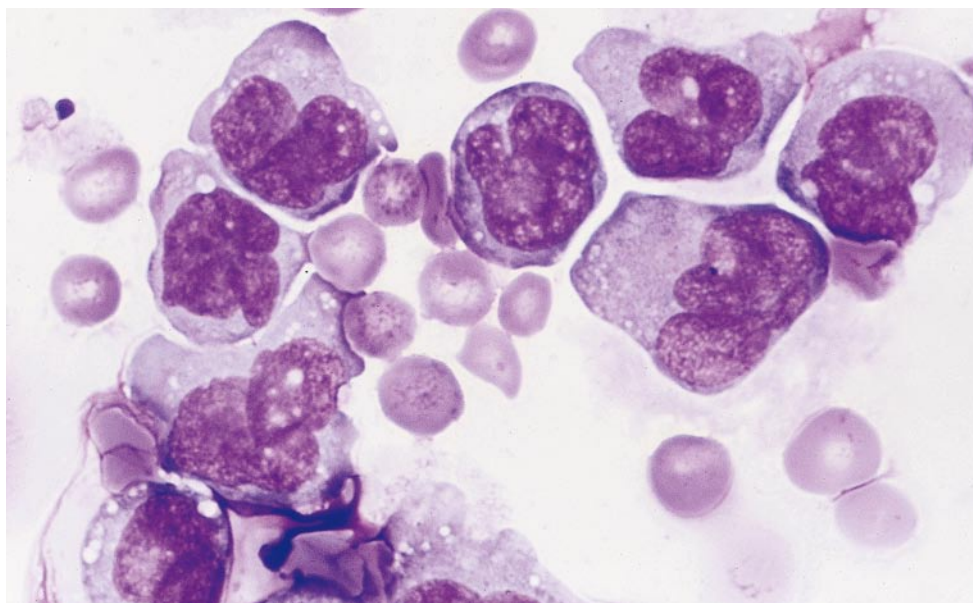


Figure 6.24 Acute myeloid leukemia (AML), M4. In cerebrospinal fluid (CSF) from patients with leukemia, it is usually sufficient simply to identify the presence of blasts; further typing (myeloid versus lymphoid and their subtypes) is done on peripheral blood and bone marrow biopsy and aspirated material. (Wright-Giemsa stain).

Malignant Lymphoma

LM occurs in 5% to 10% of patients with non-Hodgkin lymphoma, either at presentation, at a later point during the disease course, or at relapse.⁶³ Leptomeningeal involvement is more common than parenchymal brain involvement by lymphoma. Some histologic types have a higher incidence of CNS involvement than others. Diffuse large B-cell, lymphoblastic, Burkitt, and Burkitt-like lymphomas have an especially high affinity for the CNS. On the other hand, some lymphomas like Hodgkin lymphoma and small lymphocytic lymphoma are almost never seen in CSF. Patients with LM resulting from lymphoma have a higher frequency of cranial nerve signs and symptoms.⁶³

CYTOMORPHOLOGY OF LYMPHOMA IN CEREBROSPINAL FLUID:

- dispersed cells
- larger than normal lymphocytes
- irregular nuclear contours
- abnormal chromatin
- prominent nucleolus

Typically, the CSF shows a monomorphous population of highly atypical cells, with perhaps only a small percentage of normal lymphocytes (Fig. 6.25). In some cases, however, the lymphoma cells are a minority of the total cell population.

The differential diagnosis is a reactive lymphocytosis caused by meningitis. A reactive lymphocytosis is composed of a heterogeneous population of small, mature lymphocytes and larger, activated, so-called atypical lymphocytes. This distinction is not always straightforward.

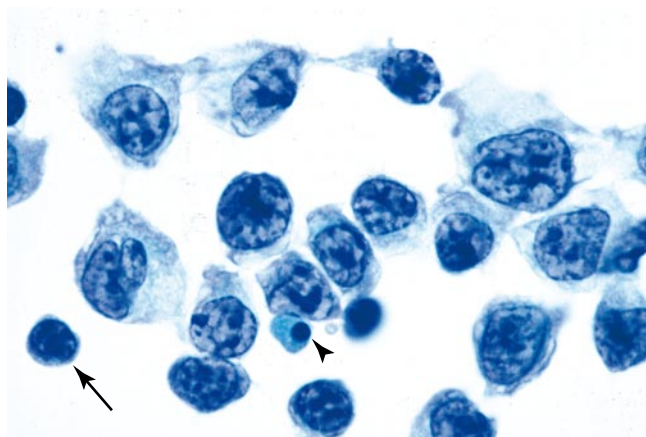


Figure 6.25 Diffuse large B-cell lymphoma. Tumor cells are dispersed as isolated cells with irregular nuclei, coarsely textured chromatin, and scant to abundant cytoplasm. Pyknosis (arrowhead) and karyorrhexis are common and can be prominent. A small, benign lymphocyte is also present (arrow; Papanicolaou stain).

Because lymphomas have many different appearances, comparison with a previous biopsy of a lymph node or other site is helpful. In some cases, definitive diagnosis is not possible without lymphoid marker studies.⁷⁴ These can be performed on duplicate air-dried cytopspins stained with antibodies against a pan B-cell marker (e.g., CD20), a pan T-cell marker (e.g., CD3), and κ and λ immunoglobulin light chains (Fig. 6.26A-C). Alternatively, fluid can be sent fresh for flow cytometric assessment of lymphocyte surface markers.^{75,76} When used selectively, successful results are obtained by flow cytometry in up to 75% of cases. In combination with cytology in the evaluation of atypical lymphoid cells in CSF, flow cytometry improves sensitivity for malignancy. Most reactive and inflammatory fluids are composed primarily of T-cells (Lyme disease is an exception), whereas the great majority of lymphomas that involve the CNS are B-cell neoplasms. Thus, a predominantly B-cell proliferation in CSF is highly suspicious for lymphoma; the diagnosis can be established by demonstrating light chain restriction (predominance of either κ or λ expression; Fig. 6.27). The polymerase chain reaction is useful in selected cases and can be performed on archival and fresh CSF samples.^{77,78}

Primary Central Nervous System Tumors

CSF cytology is an important test for documenting LM of a primary CNS tumor, either at the time of presentation or recurrence.⁷⁹ Certain CNS tumors have a greater predilection for involving the leptomeninges than others. These include medulloblastoma, ependymoma, germinoma, pineoblastoma, primitive neuroectodermal tumors (PNETs), atypical teratoid/rhabdoid tumor, choroid plexus tumors, astrocytomas and glioblastomas, and primary CNS lymphomas. The clinical features of some of these are summarized in Table 6.2.

Primary Central Nervous System Lymphoma

Primary CNS lymphoma represents between 4% and 15% of all primary CNS tumors.⁸⁰ It occurs in patients who are both immunocompetent and immunocompromised. Epstein-Barr virus (EBV) is detected in most primary CNS lymphomas in patients who are immunocompromised and almost never in those arising in individuals who are immunocompetent.⁸⁰ Diffuse large B-cell lymphoma is the most common type; the indolent small cell lymphomas and lymphomas of T-cell phenotype occur much less frequently.^{81,82} In most patients with primary CNS lymphoma, the tumor involves the brain parenchyma, with or without leptomeningeal involvement.⁸¹ But about 8% of cases involve the leptomeninges only ("primary leptomeningeal lymphoma").^{81,82}

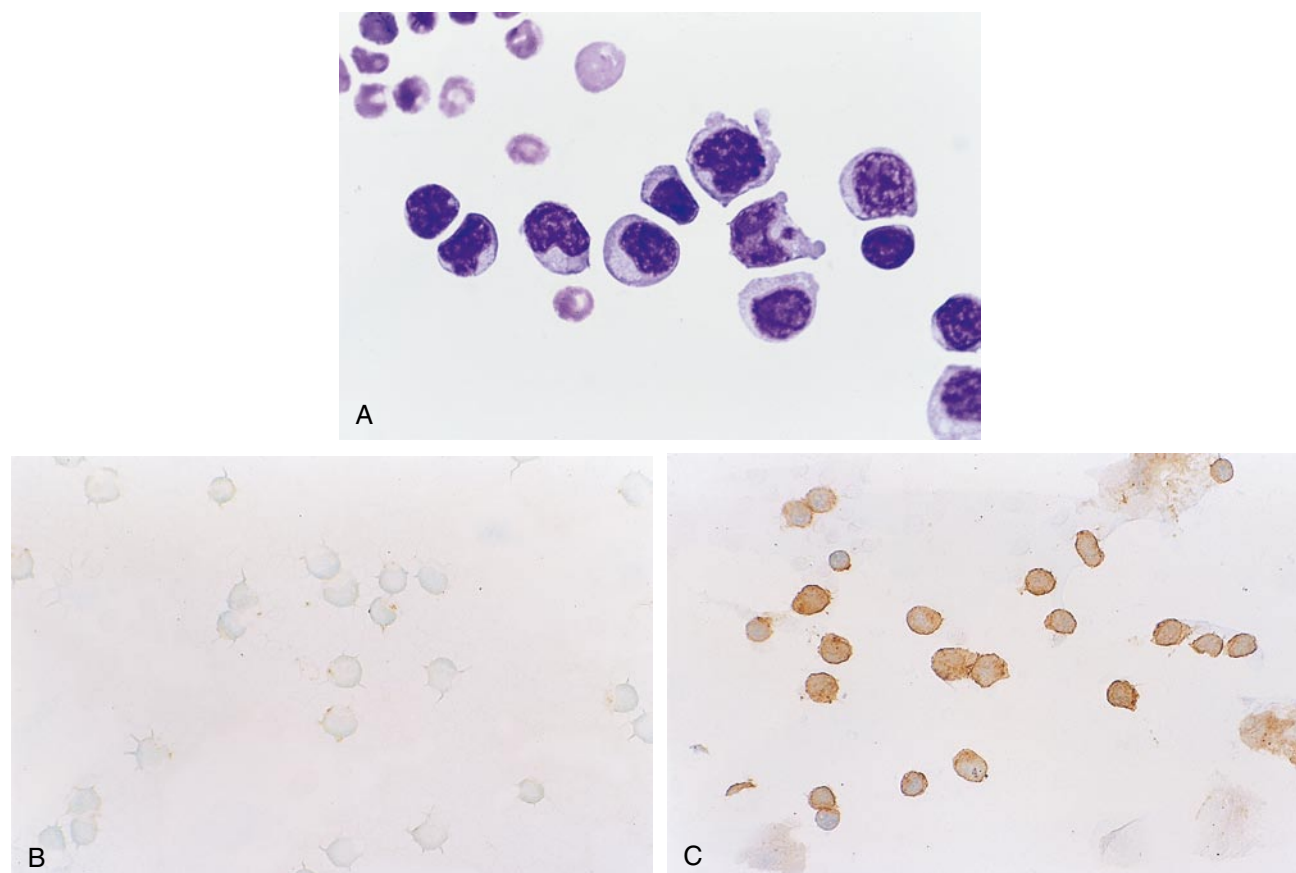


Figure 6.26 Follicular lymphoma. The malignant cells are relatively small and have irregular nuclear contours. The cells show monotypic expression of immunoglobulin light chains. A, Wright-Giemsa stain. B, κ light chain. C, λ light chain.

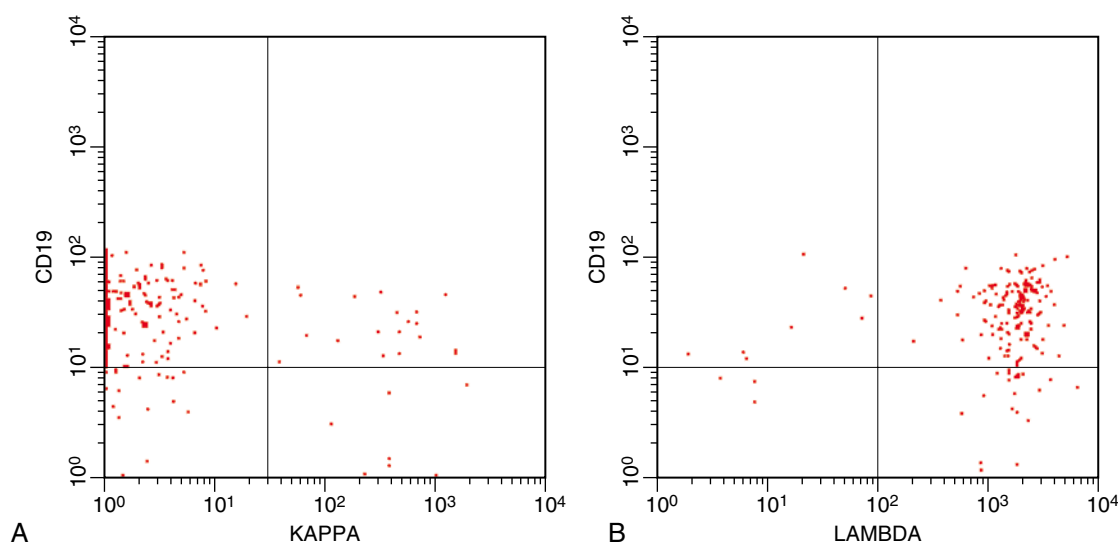


Figure 6.27 Flow cytometric analysis of cerebrospinal fluid (CSF); non-Hodgkin lymphoma. There is a population of CD19-positive B-cells that are negative for κ and positive for λ light chains.

CSF cytology is positive in one third of patients.⁸⁰ The diagnosis is difficult because in some cases the large atypical cells are outnumbered by small reactive T cells, thus mimicking the appearance of an aseptic meningitis (Fig. 6.28). Flow cytometric analysis can be

helpful by proving clonality and is highly sensitive; the technique can detect malignant cells that represent as few as 2% of the total cell population.⁷⁶ It is not practical to do flow cytometric analysis on all samples that show a lymphocytic pleocytosis, but flow cytometry

TABLE 6.2 CLINICAL FEATURES OF PRIMARY CNS TUMORS THAT SPREAD VIA CEREBROSPINAL FLUID PATHWAYS

Tumor	Predominant Age	Preferred Location(s)
Medulloblastoma	Children	Cerebellum
Glioblastoma	Adults	Cerebral hemispheres
Ependymoma	Children and adolescents	Fourth ventricle (children); spinal cord (adults)
Choroid plexus tumors	Children	Ventricles (most common: fourth)
Pineoblastoma	Children	Pineal gland
Germ cell tumors	Children and adolescents	Pineal, suprasellar regions
Atypical teratoid/rhabdoid tumor	Infants and children	Cerebellum, brainstem, cerebral hemispheres
Primary central nervous system lymphoma	Adults	Cerebral hemispheres, cerebellum, brainstem, spinal cord

should be considered in patients with suspicious clinical symptoms. Localized neurologic signs, such as cranial nerve palsies, are common in patients with primary CNS lymphoma and rare in patients with aseptic meningitis.

Medulloblastoma

Medulloblastoma is a poorly differentiated small cell tumor of uncertain histogenesis that arises in the cerebellum. It is predominantly a disease of children but is sometimes seen in adults. It tends to invade the adjacent fourth ventricle or meninges; approximately 25% of patients with medulloblastoma have positive CSF cytology (Fig. 6.29).⁸³ At autopsy, leptomeningeal

involvement is discovered in more than 50% of cases.⁸⁴ Morphologically identical tumors in the cerebrum or suprasellar region are called supratentorial primitive neuroectodermal tumors.

CYTOMORPHOLOGY OF MEDULLOBLASTOMA:

- small-sized to medium-sized cells
- hyperchromatic nucleus
- scant cytoplasm
- nucleolus may be prominent
- nuclear molding
- see Figure 6.29A and B

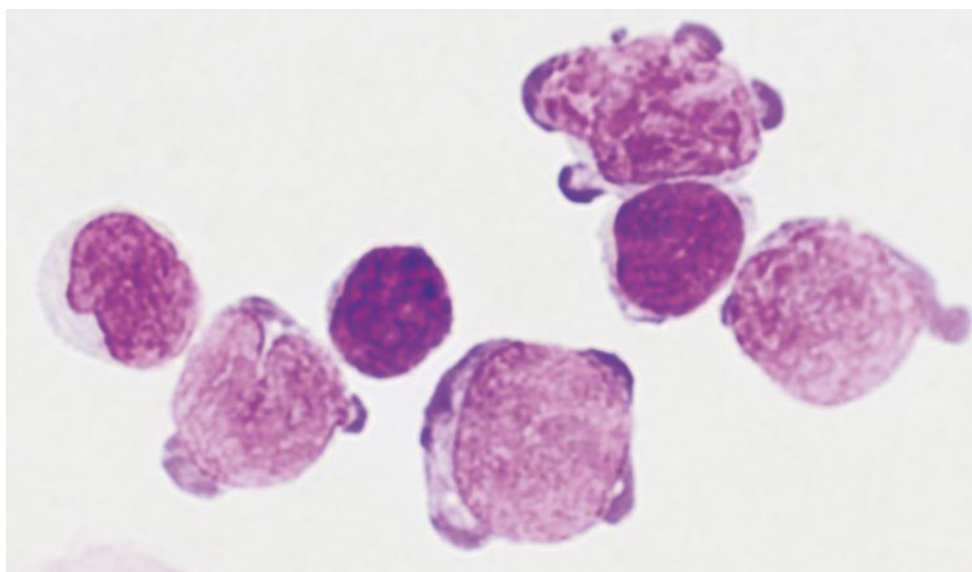


Figure 6.28 Primary central nervous system (CNS) lymphoma. Five large, atypical lymphoid cells are present in this field, one with a prominent nuclear cleave. (Two benign lymphocytes are smaller, with darker nuclei.) In some cases of primary CNS lymphoma, the malignant B-cells are outnumbered by small reactive T-cells, and the findings mimic aseptic meningitis. (Compare with Fig. 6.14B.) In this case there was a high clinical suspicion of lymphoma because the patient had cranial nerve findings (diplopia and facial droop). A portion of the sample was sent for flow cytometry, which showed immunoglobulin light chain restriction by the large cells.

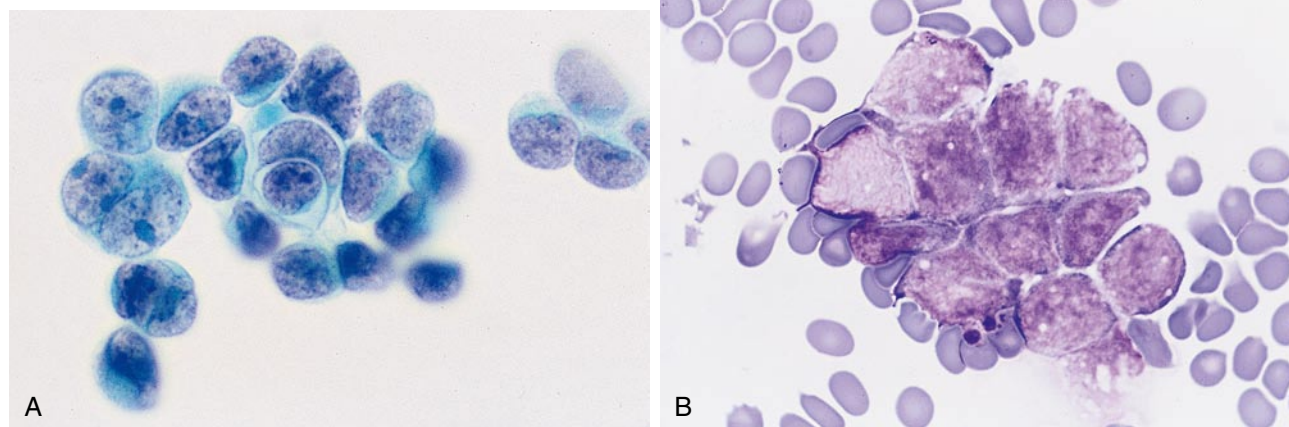


Figure 6.29 Medulloblastoma. The tumor cells are small, with hyperchromatic nuclei and scant cytoplasm. Nuclear molding is prominent. A, Papanicolaou stain. B, Wright-Giemsa stain.

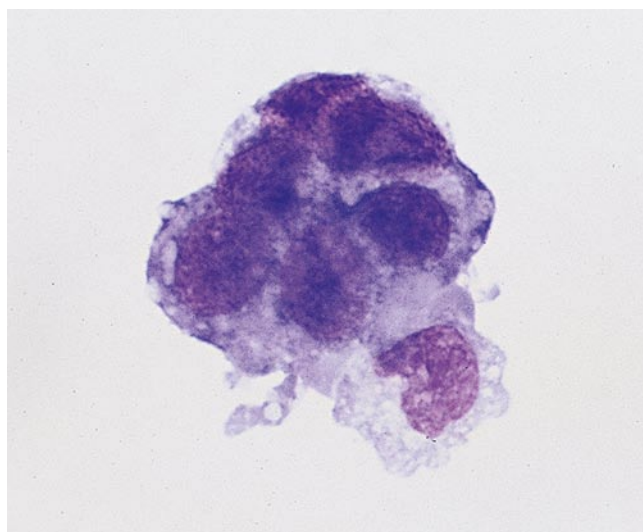


Figure 6.30 Poorly preserved lymphocytes mimicking medulloblastoma. Particularly in cerebrospinal fluid (CSF) samples older than 48 hours, lymphocytes and monocytes undergo degeneration, cluster together uncharacteristically, and even show nuclear molding (Wright-Giemsa stain).

DIFFERENTIAL DIAGNOSIS OF MEDULLOBLASTOMA:

- poorly preserved lymphocytes (see Fig. 6.30)
- germinal matrix
- small cell carcinoma
- pineoblastoma
- neuroblastoma
- retinoblastoma
- anaplastic ependymoma

When clusters of small cells are seen in a neonate, especially one born prematurely, they most likely represent cells of germinal matrix origin³⁰ (see Fig. 6.7). These cells mimic a poorly differentiated small cell malignancy like medulloblastoma. Clinical correlation is important; the

possibility of benign cells of germinal matrix origin should be considered in all neonates with hydrocephalus and in premature infants with intraventricular hemorrhage. In cases associated with hemorrhage, hemosiderin-laden macrophages are numerous. Morphologically, medulloblastoma cells are indistinguishable from those of other anaplastic small cell tumors like small cell carcinoma of the lung, but clinical and radiographic findings help in establishing the diagnosis. Anaplastic ependymoma of the fourth ventricle may be clinically and cytologically impossible to distinguish from medulloblastoma, however.

Astrocytomas and Glioblastoma

This group of tumors is the most commonly encountered primary CNS malignancy in adults. They arise from astrocytes, the supporting cells of the CNS, and are divided into diffusely infiltrating astrocytomas, pilocytic astrocytoma (WHO grade I), and a few other rare types.⁸⁵ Diffusely infiltrating astrocytomas are further subclassified as diffuse astrocytomas (WHO grade II), anaplastic astrocytomas (WHO grade III), and glioblastoma (WHO grade IV), based on the degree of nuclear pleomorphism, necrosis, and vascular proliferation. These tumors can arise virtually anywhere in the CNS (cerebrum, cerebellum, brainstem, and spinal cord), and all types can spread to the ventricles and subarachnoid space.

Glioblastoma, the highest grade astrocytoma, is the most common of all brain tumors. It comprises 12% to 15% of all intracranial tumors and more than half of all astrocytic neoplasms.⁸⁵ *Pilocytic astrocytoma* is a circumscribed, slowly growing, often cystic tumor that arises in children and young adults. Subarachnoid space involvement is a characteristic feature of pilocytic astrocytomas, and CSF is positive in 11% of patients in whom it is examined,⁸⁶ but dissemination along CSF pathways is uncommon. An uncommon type of astrocytic neoplasm, *gliomatosis cerebri*, presents as a diffuse astrocytic proliferation without an obvious tumor mass. It often spreads to involve the subarachnoid space, and CSF examination is useful in establishing a diagnosis.⁸⁷

CYTOMORPHOLOGY OF ANAPLASTIC ASTROCYTOMA AND GLIOBLASTOMA:

- large pleomorphic cells, or
- smaller, anaplastic cells with hyperchromatic nuclei

Anaplastic astrocytomas and glioblastomas appear as isolated cells and small clusters. They have hyperchromatic, highly pleomorphic nuclei with coarse chromatin, irregular nuclear outlines, and prominent nucleoli⁸⁸ (Fig. 6.31).

Pilocytic astrocytomas appear in CSF as isolated cells or clusters. The isolated cells have long, hairlike cytoplasmic processes, and clustered cells appear epithelioid, often with finely textured chromatin and cobweb-like cytoplasm.⁸⁶

Ependymoma

Ependymomas arise from the lining cells of the ventricles and can occur anywhere in the CNS, but the fourth ventricle and spinal cord are the most common sites. Although more common in children and adolescents, they are also seen in adults. Histologically, ependymomas are considered WHO grade II neoplasms and are comprised of monomorphic cells that form perivascular pseudorosettes and ependymal rosettes, and mitoses are rare. The prognosis is poor because their location makes them inoperable. Of the patients who undergo

CSF cytologic examination, 11% have either suspicious or positive cytology.⁸⁹ Clinically significant LM is uncommon, however, most likely because of the inability of the seedlings to adhere and proliferate.⁸⁴

The *myxopapillary ependymoma* is a slowly growing, WHO grade I tumor with a favorable prognosis. It accounts for 13% of all ependymomas, has a predilection for young adults, and is virtually always located at the terminal end of the spinal cord.⁸⁵ *Anaplastic ependymomas* represent the other end of the spectrum of ependymomas. Considered WHO grade III tumors, anaplastic ependymomas are poorly differentiated, with brisk mitotic activity.

CYTOMORPHOLOGY OF EPENDYMOMAS:

- isolated cells or small groups
- round, eccentrically placed nucleus

The appearance of ependymoma cells in CSF depends on the histologic subtype.⁸⁹ The cells of the usual type of ependymoma are cuboidal or columnar (Fig. 6.32), with a round or oval, bland nucleus and a moderate amount of cytoplasm.^{88,89} They can be difficult to distinguish from benign ependymal cells.^{4,90,91} In patients with a tanyctic ependymoma, CSF samples show bipolar cells with long, hairlike glial processes.⁸⁹ Anaplastic ependymomas are cytologically indistinguishable from medulloblastomas.

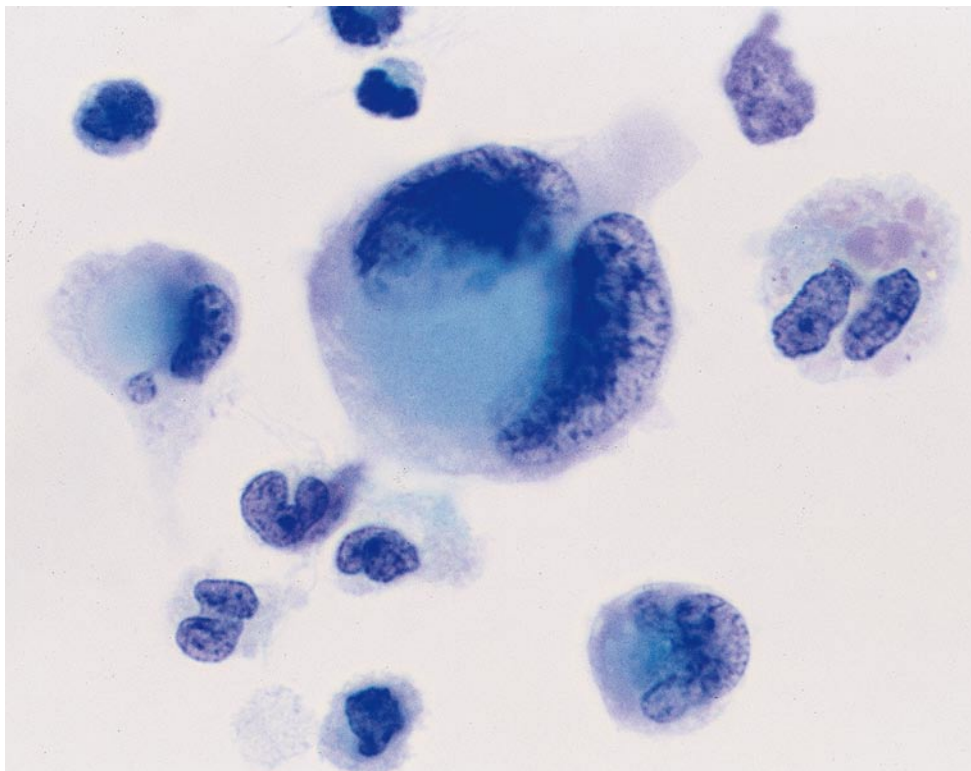


Figure 6.31 Glioblastoma. The tumor cells are highly pleomorphic, with hyperchromatic nuclei and abundant cytoplasm (Papanicolaou stain).

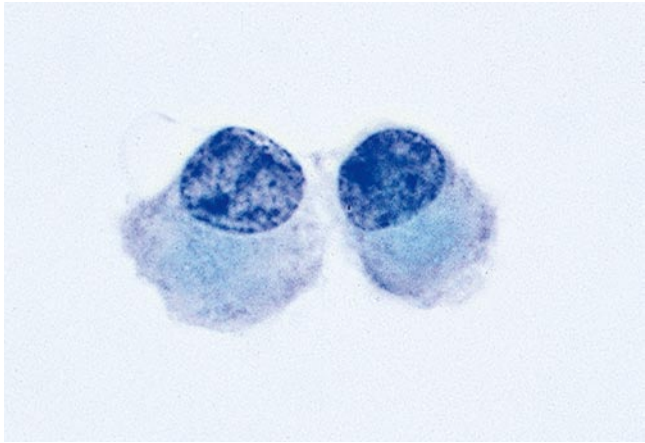


Figure 6.32 Ependymoma. The tumor cells have a round, eccentrically placed nucleus (Papanicolaou stain).

Oligodendroglioma

This tumor of oligodendrocytes is more common in adults but is also seen in children. The great majority occur in the cerebral hemisphere. Spread to the CSF can be either rapid and fatal or chronic and sustained. The tumor is composed of uniform polygonal cells with round nuclei. In tissue sections there is a pronounced perinuclear cytoplasmic clearing that imparts a characteristic “fried egg” appearance to the tumor cells. Positive CSF has been reported,^{91,92} but the cytologic features have not been described in depth.

CYTOMORPHOLOGY OF OLIGODENDROGLIOMA:

- uniform round cells
- distinct cell outlines
- abundant clear cytoplasm
- round nucleus
- fine chromatin
- prominent nucleolus

Atypical Teratoid/Rhabdoid Tumor

The atypical teratoid/rhabdoid tumor (ATRT) is a newly described CNS tumor of unknown histogenesis that predominantly affects infants and children. One third of patients have LM at presentation.⁸⁵ Histologically and cytologically, the tumor contains rhabdoid cells: medium-sized to large-sized cells with a round, eccentrically placed nucleus, and a prominent nucleolus. The cytoplasm is homogeneous and may contain a large, poorly defined, dense, inclusion-like structure that pushes aside the nucleus (Fig. 6.33). Binucleated cells can be seen. Two third of cases have a poorly differentiated small cell component that resembles medulloblastoma cells in CSF. Atypical teratoid/rhabdoid tumor cells are immunoreactive for epithelial membrane antigen

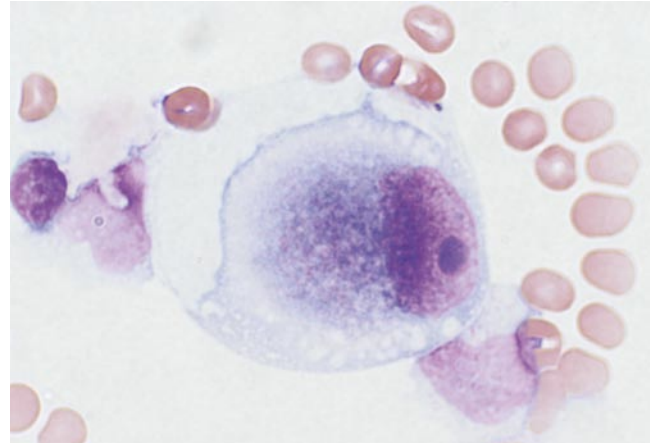


Figure 6.33 Atypical teratoid/rhabdoid tumor. This large rhabdoid cell has a dense cytoplasmic “body” that pushes the nucleus to an eccentric position. A lymphocyte, two monocytes, and some red blood cells are also present (Wright-Giemsa stain).

and vimentin, and they may express smooth muscle actin, GFAP, neurofilament protein, and keratin.

Choroid Plexus Tumors

Tumors of the choroid plexus account for 0.5% to 0.6% of intracranial tumors. Predominantly seen in children, they also occur in adults. The fourth ventricle is the most common location, followed by the lateral ventricles and the third ventricle. The great majority are cytologically benign choroid plexus papillomas. Histologically, the tumors are papillary, composed of a fibrovascular core covered by a single layer of cuboidal or columnar epithelium (Fig. 6.34).

CYTOMORPHOLOGY OF CHOROID PLEXUS PAPILLOMA:

- large clusters
- uniform cuboidal cells
- round nucleus
- see Figure 6.34

The individual cells of a choroid plexus papilloma can be indistinguishable from normal choroid plexus or ependymal cells. When present in abundance and in large clusters, the diagnosis of a papilloma is suggested.⁹³

Choroid plexus carcinomas are rare, arising almost exclusively in infants and children.⁹⁴ Their existence in adults has been questioned.⁸⁴

CYTOMORPHOLOGY OF CHOROID PLEXUS CARCINOMA:

- single cells or clusters
- pleomorphic nuclei
- prominent nucleolus
- indistinguishable from adenocarcinoma

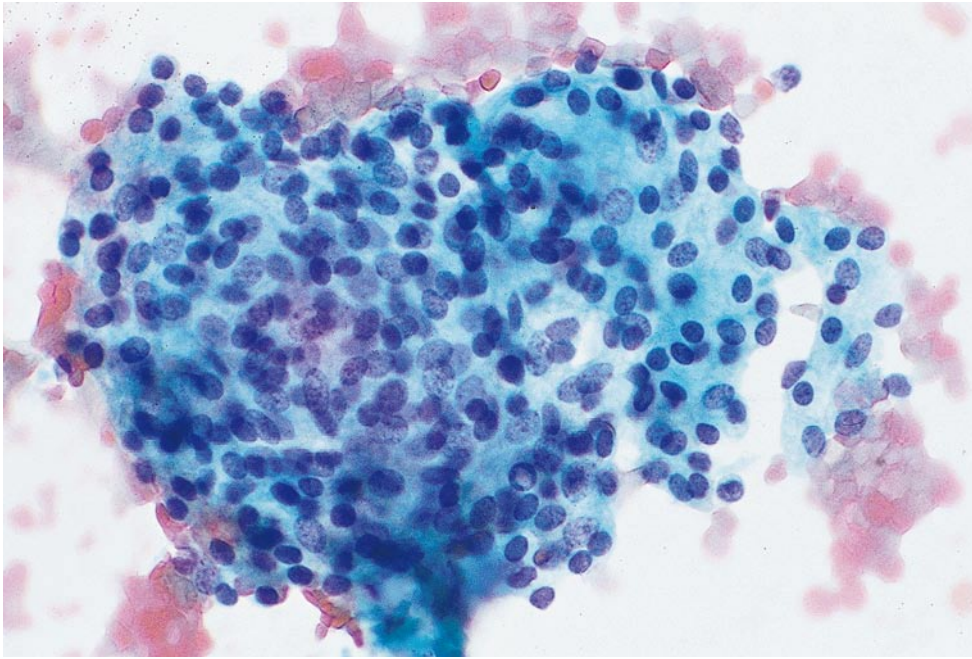


Figure 6.34 Choroid plexus papilloma. Tumor cells are arranged in large, three-dimensional clusters of uniform cuboidal cells with a round or oval nucleus (Papanicolaou stain).

In adults, a metastasis from an occult lung adenocarcinoma must be excluded clinically before the diagnosis can be considered.

Pineal Tumors

The pineal gland is a small, midline structure whose precise function is not known. Pineal tumors are rare, accounting for 0.4% to 1% of intracranial tumors. More than half of the tumors that arise here are germ cell tumors. The rest arise from astrocytes or from specialized neurons called pinealocytes. Tumors of pinealocytic origin affect children more often than adults. They are subclassified as pineocytomas, pineoblastomas, and pineal parenchymal tumors of intermediate differentiation. Pineoblastomas commonly spread to the CSF; in a study of 11 cases, all showed leptomeningeal involvement at autopsy.⁹⁵ Pineoblastoma is morphologically indistinguishable from medulloblastoma.

Pineocytomas are typically localized neoplasms that do not metastasize. Although aggressive behavior has been described,⁹⁶ some authors question whether these aggressive tumors might have been misclassified.⁸⁵

Germ Cell Tumors

Germ cell neoplasms in the brain arise from primordial germ cells that migrated to the CNS, particularly to the pineal and suprasellar areas. The entire spectrum of testicular and ovarian germ cell tumors can be seen. The most common is the germinoma, histologically identical to the testicular seminoma and the ovarian dysgerminoma and equally radiosensitive. It occurs most commonly in children and young adults, and is more common in

males than females. It is likely to infiltrate ventricles and meninges and spread via the CSF (Fig. 6.35).⁹⁷⁻⁹⁹ In one study, two of seven patients had positive cytology on CSF examination.¹⁰⁰ Serum or CSF elevations of alpha-fetoprotein, beta-HCG, and placental alkaline phosphatase are considered strong presumptive evidence in patients suspected of harboring a CNS germ cell tumor.

CYTOMORPHOLOGY OF GERMINOMA:

- isolated cells
- large, round nucleus
- prominent nucleolus
- moderate amount of cytoplasm
- see Figure 6.35

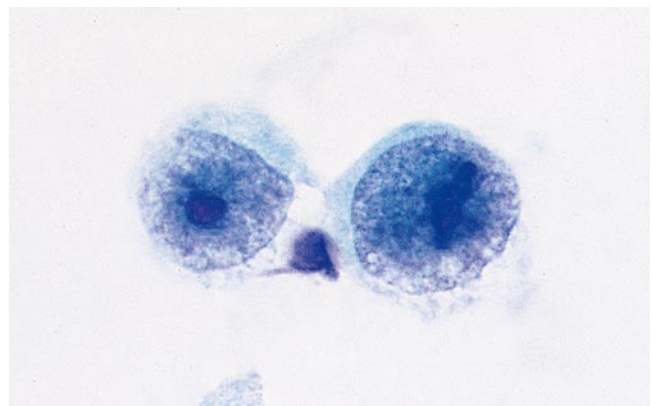


Figure 6.35 Germinoma. The cells are large, with a round nucleus and a prominent nucleolus (Papanicolaou stain).

Other types of germ cell tumors, including embryonal carcinoma, endodermal sinus tumor, teratoma, choriocarcinoma, and various combinations of these tumors can also occur, and can be identified in CSF.¹⁰¹ Choriocarcinomas are highly chemosensitive, and early diagnosis is essential for the prompt institution and success of therapy.¹⁰²

Other Tumors of the Central Nervous System

Some of the common tumors of the CNS rarely spread by CSF pathways and are therefore rarely diagnosed by CSF cytology. Meningiomas constitute 14% of intracranial tumors and almost all are benign. Their spread by means of CSF is extremely uncommon¹⁰³ and probably not distinguishable by cytologic methods.^{19,90} Pituitary adenomas represent up to 25% of intracranial tumors. Most are benign, but a small percentage spread to the CSF. In a study of 20 cases, the CSF was positive in both of the patients whose tumors behaved aggressively.¹⁰⁴ Exfoliated cells are arranged in clusters that mimic metastatic adenocarcinoma.⁴

REFERENCES

1. Quincke H: Die Lumbarpunktion des Hydrocephalus. *Klin Wochenschr* 1891;28:929-933, 965-968.
2. Wynter W: Four cases of tubercular meningitis in which paracentesis of the theca vertebralis was performed for the relief of fluid pressure. *Lancet* 1891;1:981-982.
3. Dufour H: Méningite sarcomateuse diffuse avec envahissement de la Moelle et des Racines: Cytologie positive et spéciale du liquide céphalorachidien. *Rev Neurol* 1904;12:104-106.
4. Bigner SH, Johnston WW: *Cytopathology of the Central Nervous System*. Chicago, American Society of Clinical Pathologists Press, 1994.
5. Rosenthal DL: *Cytology of the Central Nervous System*. Basel, Switzerland S Karger, 1984.
6. Torzewski M, Lackner K, Bohl J, Sommer C: *Integrated Cytology of Cerebrospinal Fluid*. New York, Springer, 2008.
7. Kjeldsberg CR, Knight JA: *Body Fluids: Laboratory Examination of Amniotic, Cerebrospinal, Seminal, Serous, and Synovial Fluids*, 3rd ed. Chicago, American Society of Clinical Pathologists Press, 1993.
8. Fishman RA: *Cerebrospinal Fluid in Diseases of the Nervous System*, 2nd ed. Philadelphia, WB Saunders, 1992.
9. Barshes N, Demopoulos A, Engelhard HH: Anatomy and physiology of the leptomeninges and CSF space. *Cancer Treat Res* 2005;125:1-16.
10. Rogers LR, Duchesneau PM, Nunez C, et al.: Comparison of cisternal and lumbar CSF examination in leptomeningeal metastasis. *Neurology* 1992;42(6):1239-1241.
11. NCCLS: *Nongynecologic Cytologic Specimens: Collection and Cytopreparatory Techniques; Approved Guideline*. Wayne, Penn, NCCLS, 1999.
12. Glantz MJ, Cole BF, Glantz LK, et al.: Cerebrospinal fluid cytology in patients with cancer: Minimizing false-negative results. *Cancer* 1998;82(4):733-739.
13. Sciarra D, Carter S: Lumbar puncture headache. *JAMA* 1952;148:841-842.
14. Ahmed SV, Jayawarna C, Jude E: Post lumbar puncture headache: Diagnosis and management. *Postgrad Med J* 2006;82(973):713-716.
15. Breuer AC, Tyler HR, Marzewski DJ, Rosenthal DS: Radicular vessels are the most probable source of needle induced blood in lumbar puncture: Significance for the thrombocytopenic cancer patient. *Cancer* 1982;49:2168-2172.
16. Rohlfing MB, Barton TK, Bigner SH, Johnston WW: Contamination of cerebrospinal fluid specimens with hematogenous blasts in patients with leukemia. *Acta Cytol* 1981;25:611-615.
17. Prayson RA, Fischler DF: Cerebrospinal fluid: An 11-year experience with 5951 specimens. *Arch Pathol Lab Med* 1998;122:47-57.
18. Glass JP, Melamed M, Chernik NL, Posner JB: Malignant cells in cerebrospinal fluid (CSF): The meaning of a positive CSF cytology. *Neurology* 1979;29:1369-1375.
19. Gondos B, King EB: Cerebrospinal fluid cytology: Diagnostic accuracy and comparison of different techniques. *Acta Cytol* 1976;20:542-547.
20. Wasserstrom WR, Glass JP, Posner JB: Diagnosis and treatment of leptomeningeal metastases from solid tumors: Experience with 90 patients. *Cancer* 1982;49:759-772.
21. Kaplan JG, DeSouza TG, Farkash A, et al.: Leptomeningeal metastases: Comparison of clinical features and laboratory data of solid tumors, lymphomas and leukemias. *J Neurooncol* 1990;9(3):225-229.
22. Price RA, Johnson WW: The central nervous system in childhood leukemia: I. The arachnoid. *Cancer* 1973;31:520-533.
23. Wertlake PT, Markovits BA, Stellar S: Cytologic evaluation of cerebrospinal fluid with clinical and histologic correlation. *Acta Cytol* 1972;6:229-239.
24. Borowitz M, Bigner SH, Johnston WW: Diagnostic problems in the cytologic evaluation of cerebrospinal fluid for lymphoma and leukemia. *Acta Cytol* 1981;25:665-674.
25. Davies SE, Gormus BJ, Yarchoan R, Kaplan ME: Cryptococcal meningitis with false-positive cytology in the CSF: Use of T-cell rosetting to exclude meningeal lymphoma. *JAMA* 1978;22:2369-2370.
26. Szyfelbein WM, Ross JS: Lyme disease meningopolyneuritis simulating malignant lymphoma. *Mod Pathol* 1988;1:464-468.
27. Health and Public Policy Committee, American College of Physicians: The diagnostic spinal tap. *Ann Intern Med* 1986;104:880-885.
28. Dyken PR: Cerebrospinal fluid cytology: Practical clinical usefulness. *Neurology* 1975;25:210-217.
29. De Reuck J, Vanderdonck P: Choroid plexus and ependymal cells in CSF cytology. *Clin Neurol Neurosurg* 1986;88:177-179.
30. Fischer JR, Davey DD, Gulley ML, Goeken JA: Blast-like cells in cerebrospinal fluid of neonates: Possible germinal matrix origin. *Am J Clin Pathol* 1989;91:255-258.
31. Jaffey PB, Varma SK, DeMay RM, et al.: Blast-like cells in the cerebrospinal fluid of young infants: Further characterization of clinical setting, morphology, and origin. *Am J Clin Pathol* 1996;105:544-547.
32. Chen KT, Moseley D: Cartilage cells in cerebrospinal fluid. *Arch Pathol Lab Med* 1990;114:212.
33. Kruskall MS, Carter SR, Ritz LP: Contamination of cerebrospinal fluid by vertebral bone marrow cells during lumbar puncture. *N Engl J Med* 1983;697-700.
34. Leiman G, Klein C, Berry AV: Cells of nucleus pulposus in cerebrospinal fluid. A case report. *Acta Cytol* 1980;24:347-349.
35. De Reuck J, Vanderdonck P, de Bleecker J, et al.: Mitotic activity in cerebrospinal fluid cells. *Clin Neurol Neurosurg* 1988;90:117-119.
36. Zeman D, Adam P, Kalistova H, et al.: Cerebrospinal fluid cytologic findings in multiple sclerosis. A comparison between patient subgroups. *Acta Cytol* 2001;45:51-59.
37. Granter SR, Doolittle MH, Renshaw AA: Predominance of neutrophils in the cerebrospinal fluid of AIDS patients with cytomegalovirus radiculopathy. *Am J Clin Pathol* 1996;105:364-346.

38. Kuberski T: Eosinophils in the cerebrospinal fluid. *Ann Intern Med* 1979;91:70-75.
39. Gupta PK, Gupta PC, Roy S, Banerji AK: Herpes simplex encephalitis, cerebrospinal fluid cytology studies: two case reports. *Acta Cytol* 1972;16:563-565.
40. Tedder DG, Ashley R, Tyler KL, Levin MJ: Herpes simplex virus infection as a cause of benign recurrent lymphocytic meningitis. *Am Intern Med* 1994;121:334-338.
41. Yamamoto LJ, Tedder DG, Ashley R, Levin MJ: Herpes simplex virus type I DNA in cerebrospinal fluid of a patient with Mollaret's meningitis. *N Engl J Med* 1991;325:1082-1085.
42. Chan TY, Parwani AV, Levi AW, Ali SZ: Mollaret's meningitis: cytopathologic analysis of fourteen cases. *Diagn Cytopathol* 2003;28(5):227-231.
43. Ross JS, Magro C, Szyfelbein W, Sorensen S: Cerebrospinal fluid pleocytosis in aseptic meningitis: cytomorphic and immunocytochemical features. *Diagn Cytopathol* 1991;532-535.
44. Bonnín JM, Garcia JH: Primary malignant non-Hodgkin's lymphoma of the central nervous system. *Pathol Ann* 1987;22:353-375.
45. Walts AE: Cerebrospinal fluid cytology: selected issues. *Diagn Cytopathol* 1992;8:394-408.
46. Brogi E, Cibas ES: Cytologic detection of *Toxoplasma gondii* tachyzoites in cerebrospinal fluid. *Am J Clin Pathol* 2000;114:951-955.
47. Weller PF, Liu LX: Eosinophilic meningitis. *Semin Neurol* 1993;13:161-168.
48. Duma RJ, Ferrell HW, Nelson EC, Jones MM: Primary amebic meningoencephalitis. *N Engl J Med* 1969;281:1315-1323.
49. DeAngelis LM, Boutros D: Leptomeningeal metastasis. *Cancer Invest* 2005;23(2):145-154.
50. Chamberlain MC: Neoplastic meningitis. *Semin Neurol* 2004;24(4):363-374.
51. Enting RH: Leptomeningeal neoplasia: Epidemiology, clinical presentation, CSF analysis and diagnostic imaging. *Cancer Treat Res* 2005;125:17-30.
52. Bigner SH, Johnston WW: The diagnostic challenge of tumors manifested initially by the shedding of cells into cerebrospinal fluid. *Acta Cytol* 1984;28:29-36.
53. Csako G, Chandra P: Bronchioloalveolar carcinoma presenting with meningeal carcinomatosis: Cytologic diagnosis in cerebrospinal fluid. *Acta Cytol* 1986;30:653-656.
54. Ringenbergs QS, Francis R, Doll DC: Meningeal carcinomatosis as the presenting manifestation of tumors of unknown origin. *Acta Cytol* 1990;34:590-592.
55. Scully RE, Mark EJ, McNeely WF, McNeely BU (eds): Case records of the Massachusetts General Hospital. Case 321987. *N Engl J Med* 1987;317:366-375.
56. Boogerd W, Vroom TM, Van Heerde P, et al.: CSF cytology versus immunocytochemistry in meningeal carcinomatosis. *J Neurol Neurosurg Psych* 1988;51:142-145.
57. Hajdu SI, Ashton PR, Carter D, et al.: Diagnostic cytology seminar. *Acta Cytol* 1982;26:874-876.
58. Wechsler LR, Gross RA, Miller DC: Meningeal gliomatosis with "negative" CSF cytology: the value of GFAP staining. *Neurology* 1984;34:1611-1615.
59. Trojanowski JQ, Atkinson B, Lee VM: An immunocytochemical study of normal and abnormal human cerebrospinal fluid with monoclonal antibodies to glial fibrillary acidic protein. *Acta Cytol* 1986;30:235-239.
60. Aichner F, Schuler G: Primary leptomeningeal melanoma: Diagnosis by ultrastructural cytology of cerebrospinal fluid and cranial computed tomography. *Cancer* 1982;50:1751-1756.
61. Schmidt P, NeuenJacob E, Blanke M, et al.: Primary malignant melanoblastosis of the meninges: Clinical, cytologic and neuropathologic findings in a case. *Acta Cytol* 1988;32:713-718.
62. Borowitz MJ, DiGiuseppe JA: Acute lymphoblastic leukemia. In Knowles DM (ed) *Neoplastic Hematopathology*. Philadelphia, Lippincott, Williams & Wilkins, 2001, pp. 1643-1665.
63. Nolan CP, Abrey LE: Leptomeningeal metastases from leukemias and lymphomas. *Cancer Treat Res* 2005;125:53-69.
64. Jandl JH: *Blood: Textbook of Hematology*. Boston, Little, Brown, 1996.
65. Meyer RJ, Ferreira PP, Cuttner J, et al.: Central nervous system involvement at presentation in acute granulocytic leukemia: A prospective cytocentrifuge study. *Am J Med* 1980;68:691-694.
66. Brunning RD: Acute myeloid leukemia. In Knowles DM (ed): *Neoplastic Hematopathology*. Philadelphia, Lippincott, Williams & Wilkins, 2001, pp. 1667-1715.
67. Vardiman JW, Harris NL, Brunning RD: The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood* 2002;100(7):2292-2302.
68. Jaffe ES, Harris NL, Stein M, Vardiman JW (eds): *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours and Haematopoietic and Lymphoid Tissues*. Lyon, France, IARC Press, 2001.
69. Gétaz EP, Miller GJ: Spinal cord involvement in chronic lymphocytic leukemia. *Cancer* 1979;43:1858-1861.
70. Morrison C, Shah S, Flinn IW: Leptomeningeal involvement in chronic lymphocytic leukemia. *Cancer Pract* 1998;6(4):223-228.
71. Liepman MK, Votaw ML: Meningeal leukemia complicating chronic lymphocytic leukemia. *Cancer* 1981;47:2482-2484.
72. Boogerd W, Vroom TM: Meningeal involvement as the initial symptom of B cell chronic lymphocytic leukemia. *Eur Neurol* 1986;25:461-464.
73. Schwartz JH, Canellos GP, Young RC, DeVita VT Jr: Meningeal leukemia in the blastic phase of chronic granulocytic leukemia. *Am J Med* 1975;9:819-828.
74. Li CY, Witzig TE, Philylyk RL, et al.: Diagnosis of B-cell non-Hodgkin's lymphoma of the central nervous system by immunocytochemical analysis of cerebrospinal fluid lymphocytes. *Cancer* 1986;57:737-744.
75. Finn WG, Peterson LC, James C, Goolsby CL: Enhanced detection of malignant lymphoma in cerebrospinal fluid by multiparameter flow cytometry. *Am J Clin Pathol* 1988;110:3416.
76. French CA, Dorfman DM, Shaheen G, Cibas ES: Diagnosing lymphoproliferative disorders involving the cerebrospinal fluid: Increased sensitivity using flow cytometric analysis. *Diagn Cytopathol* 2000;23:369-374.
77. Shibata D, Nichols P, Sherrod A, et al.: Detection of occult CNS involvement of follicular small cleaved lymphoma by the polymerase chain reaction. *Mod Pathol* 1990;3:71-75.
78. Mattu R, Sorbara L, Filie AC, et al.: Utilization of polymerase chain reaction on archival cytologic material: A comparison with fresh material with special emphasis on cerebrospinal fluids. *Mod Pathol* 2004;17(10):1295-1301.
79. Packer RJ, Siegel KR, Sutton LN, et al.: Leptomeningeal dissemination of primary central nervous system tumors of childhood. *Ann Neurol* 1985;18:217-221.
80. Nuckols JD, Liu K, Burchette J, et al.: Primary central nervous system lymphomas: A 30 year experience at a single institution. *Mod Pathol* 1999;12:1167-1173.
81. Shenkier TN: Unusual variants of primary central nervous system lymphoma. *Hematol Oncol Clin North Am* 2005;19(4):651-664, vi.
82. Lachance DH, O'Neill BP, Macdonald DR, et al.: Primary leptomeningeal lymphoma: Report of 9 cases, diagnosis with immunocytochemical analysis, and review of the literature. *Neurology* 1991;41(1):95-100.
83. Jereb B, Reid A, Ahuja RK: Patterns of failure in patients with medulloblastoma. *Cancer* 1982;50:2941-2947.
84. Russel DS, Rubinstein LJ: *Pathology of Tumours of the Nervous System*, 5th ed. Baltimore, Williams & Wilkins, 1989.

85. Kleihues P, Cavenee W (eds): Pathology and genetics of tumours of the nervous system. Lyon, France, IARC Press, 2000.
86. Browne TJ, Goumnerova LC, De Girolami U, Cibas ES: Cytologic features of pilocytic astrocytoma in cerebrospinal fluid specimens. *Acta Cytol* 2004;48(1):3-8.
87. Miller RR, Lin F, Mallonee MM: Cytologic diagnosis of gliomatosis cerebri. *Acta Cytol* 1981;25:37-39.
88. Chhieng DC, Elgert P, Cohen JM, et al.: Cytology of primary central nervous system neoplasms in cerebrospinal fluid specimens. *Diagn Cytopathol* 2002;26(4):209-212.
89. Qian X, Cibas ES, Goumnerova L, De Girolami U: Cerebrospinal fluid findings in patients with ependymal neoplasms: A bi-institutional retrospective study of 50 cases. *Acta Cytol* 2003;47:826-827.
90. Naylor B: The cytologic diagnosis of cerebrospinal fluid. *Acta Cytol* 1964;8:141-149.
91. Watson CW, Hajdu SI: Cytology of primary neoplasms of the central nervous system. *Acta Cytol* 1977;21:40-47.
92. Balhuizen JC, Bots GT, Schaberg A, Bosman FT: Value of cerebrospinal fluid cytology for the diagnosis of malignancies in the central nervous system. *J Neurosurg* 1978;48:747-753.
93. Buchino JJ, Mason KG: Choroid plexus papilloma: report of a case with cytologic differential diagnosis. *Acta Cytol* 1992;36:95-97.
94. Kim K, Greenblatt SH, Robinson MG: Choroid plexus carcinoma: report of a case with cytopathologic differential diagnosis. *Acta Cytol* 1985;29:846-849.
95. Herrick MK, Rubinstein LJ: The cytological differentiating potential of pineal parenchymal neoplasms (true pinealomas): A clinicopathological study of 28 tumors. *Brain* 1979;102:289-320.
96. D'Andrea AD, Packer RJ, Rorke LB, et al.: Pineocytomas of childhood: A reappraisal of natural history and response to therapy. *Cancer* 1987;59:1353-1357.
97. Gindhart TD, Tsukahara YC: Cytologic diagnosis of pineal germinoma in cerebrospinal fluid and sputum. *Acta Cytol* 1979;23:341-346.
98. Stefanko SZ, Talermin A, Mackay WM, Vuzevski VD: Infundibular germinoma. *Acta Neurochir* 1979;50:71-78.
99. Takeuchi J, Handa H, Nagata I: Suprasellar germinoma. *J Neurosurg* 1978;49:41-48.
100. DeGirolami U, Schmidek H: Clinicopathological study of 53 tumors of the pineal region. *J Neurosurg* 1973;9:455-462.
101. Kamiya M, Tateyama H, Fujiyoshi Y, et al.: Cerebrospinal fluid cytology in immature teratoma of the central nervous system: A case report. *Acta Cytol* 1991;35:757-760.
102. Page R, Doshi B, Sharr MM: Primary intracranial choriocarcinoma. *J Neurol Neurosurg Psych* 1986;49:93-95.
103. Ludwin SK, Conley FK: Malignant meningioma metastasizing through the cerebrospinal pathways. *J Neurol Neurosurg Psych* 1975;38:136-142.
104. Ogilvy KM, Jakubowski J: Intracranial dissemination of pituitary adenomas. *J Neurol Neurosurg Psych* 1973;36:199-205.

Gastrointestinal Tract

DAVID W. KINDELBERGER and HELEN H. WANG

CLINICAL INDICATIONS

Sampling a Wider Area and Reaching Deep Organs

Better Recognition of Lymphoid Cells
Less Invasiveness
Shorter Turnaround Time

SAMPLE COLLECTION AND PROCESSING

Sample Collection
Processing the Sample

ACCURACY

REVIEW OF MORPHOLOGIC FINDINGS

ESOPHAGUS

Infections
Epithelial Repair
Barrett's Esophagus
Dysplasia in Barrett's Esophagus

Adenocarcinoma of the Esophagus
Squamous Cell Carcinoma of the Esophagus
Uncommon Tumors of the Esophagus

STOMACH

Infections
Epithelial Repair
Dysplasia and Gastric Adenomas
Adenocarcinoma of the Stomach
Endocrine Tumors (Including Carcinoid Tumor)
Non-Hodgkin Lymphoma
Gastrointestinal Stromal Tumor

DUODENUM

Infections
Adenoma and Adenocarcinoma

COLON

THE ANAL PAP TEST

The advent of improved sampling and visualization devices has cemented gastrointestinal (GI) cytology as a valuable method for the detection of malignancies, pre-malignant lesions, and infections. Direct visualization of the mucosa, coupled with imaging of submucosal structures, allows for the collection of mucosal brushings, submucosal fine-needle aspirates, and mucosal biopsies, all with a single procedure.¹ Indeed, GI cytology is increasingly seen as a rapid, efficient, and cost-effective means for the evaluation of a variety of GI tract lesions.¹

CLINICAL INDICATIONS

Clinical indications:

- suspected malignancy
- screening for dysplasia (e.g., Barrett's esophagus)
- suspected infection

A suspected malignancy is by far the most common and important indication for cytologic examination. With the aid of direct mucosal visualization, brush cytology is complementary to biopsy for detecting adenocarcinoma and dysplasia (and infections).¹ Cytologic sampling has the following advantages over biopsy.

Sampling a Wider Area and Reaching Deep Organs

Often, multiple biopsy specimens must be taken to ensure adequate sampling. In contrast, cytologic sampling, with its wider coverage area, is particularly useful for preinvasive neoplastic lesions and high-grade dysplasia, in which only a poorly defined area of irregular, rough, or vaguely nodular mucosa is seen rather than an obvious gross lesion.²⁻⁴ For this reason, cytologic sampling is ideal for the surveillance of dysplasia in patients at increased risk of squamous or adenocarcinoma of the esophagus.^{5,6} Cytology is also superior to biopsy for stenotic lesions, such as tumors of the gastric cardia or pylorus, which cannot be reached easily with biopsy forceps.¹ In addition, brush cytology specimens are more sensitive than biopsies in detecting fungal infections.⁷

Better Recognition of Lymphoid Cells

Small endoscopic biopsies are commonly distorted and squeezed. A properly prepared cytologic smear, on the other hand, yields well-preserved, isolated lymphoid cells that are easier to recognize and interpret than the

crushed, distorted cells on small biopsies. Cytologic features alone are often sufficient to render a definitive diagnosis of a large cell lymphoma.^{1,8} Two types of lymphoma pose special problems in diagnosis: a low-grade lymphoma arising from mucosa-associated lymphoid tissue and CD30-positive anaplastic large cell lymphoma.⁸ The former needs to be distinguished from a benign lymphoid infiltrate, and the latter mimics a large cell carcinoma (LCC) because the cells can be cohesive and are positive for epithelial membrane antigen (EMA) and not for leukocyte common antigen (LCA). A high level of awareness and a panel of marker studies are usually needed in such cases before a definitive diagnosis can be made.

Less Invasiveness

Obtaining a brushing sample is less traumatic than taking a grasp biopsy. This is particularly relevant for patients with clotting abnormalities and for those with vascular tumors. A diagnosis can be made on cytologic material in such patients while avoiding the risks associated with a biopsy.

Shorter Turnaround Time

Once smears have been made, they can either be air-dried and stained with a Romanowsky-type stain or immediately fixed in alcohol and stained with the toluidine blue-eosin or a modified Papanicolaou stain.^{9,10} As a result, the slides are available for interpretation in a few minutes. Although a short turnaround time may not be required in most cases, it can be crucial when urgent decisions concerning patient management need to be made.

SAMPLE COLLECTION AND PROCESSING

Various instruments and techniques for collecting material have been developed—and abandoned—over the years. The most common today is the direct brushing of a visible lesion via a fiberscope. Lesions confined to the lamina propria (e.g., signet ring cell carcinoma) or the submucosa and muscularis (e.g., GI stromal tumor, endocrine tumor, and lymphoma), however, can be difficult to sample by brush cytology. For such lesions, endoscopic fine-needle aspiration (FNA) enables sampling of lesions deep beneath necrotic debris or normal mucosa.¹¹⁻¹⁶ Endoscopic FNA is further enhanced by ultrasound guidance (endosonography). This allows the needle to reach even deeper lesions that are extrinsic to the GI tract but impinging on the lumen, like a metastatic malignancy.¹⁷ Endosonography is useful for identifying the extent of the lesion and detailing regional

anatomy to permit an assessment of the safest and most appropriate site for FNA.¹⁷

Patients with an increased risk of esophageal adenocarcinoma or squamous cell carcinoma can be surveyed periodically by nonendoscopic sampling with balloon or encapsulated samplers. These have the advantages of sampling the entire esophagus at low cost.^{5,6,18,19} Three modes of collection have been designed for this purpose: esophageal balloon cytology, encapsulated sponge cytology, and encapsulated sponge-mesh cytology.^{20,21} The esophageal balloon cytology sampler consists of a disposable, single-lumen plastic catheter attached to an expandable latex balloon. When inflated with 15 to 20 mL of air, it turns into a near-perfect sphere with a diameter of 35 mm. The other two samplers consist of a gelatin capsule attached to a flexible plastic stylet. The gelatin capsule contains a compressed polyurethane sponge in the sponge sampler and a polyurethane sponge covered with a cotton mesh in the sponge-mesh sampler. Although all three samplers obtain satisfactory yields of squamous and glandular cells, it is easier for patients to accept the encapsulated samplers. These are still in clinical trials and are not available on a routine basis.

Sample Collection

To obtain a brushing specimen, a brush is enclosed inside a transparent Teflon sheath and then passed through the endoscope. When a lesion is visualized, the brush is expelled from the sheath and firmly and briskly plunged into the mucosa 5 to 10 times. To ensure an adequate sample, the lamina propria should be penetrated. After the sample has been obtained, the brush is retracted into its Teflon sheath, removed from the patient, extruded from the sheath again, and rinsed in a preservative solution for liquid-based preparations or pressed against glass slides as direct smears.

The endoscopic FNA involves introducing the needle through the biopsy channel of a fiberoptic endoscope. The lesion is localized either by direct vision or by ultrasound. Once the needle is in the lesion, negative pressure is applied to the needle using a 10- or 20-mL syringe. With the negative pressure maintained, the needle is moved back and forth within the lesion. Finally, the pressure is released, the needle removed from the scope channel, and the material prepared in the usual way for cytologic examination.

To obtain exfoliative cytology of the esophagus non-endoscopically, the patient swallows a sampler of choice. Once the sampler reaches the stomach, it can be inflated, as with the balloon sampler, or a sponge exposed by waiting 10 minutes for gelatin to dissolve. Epithelial cells are collected on the sampler when it is pulled out of the patient.

Processing the Sample

The needle or brush is rinsed in transport/preservative medium (buffered saline solution, 50% ethanol, CytoLyt®, or CytoRich®).^{1,22,23} Alternatively, the material on the brush, needle, or sampler is rolled or spread onto one or more clean slide(s). The slide is immersed immediately in 95% alcohol for fixation. The importance of immediate fixation cannot be overemphasized because cells that are thinly spread out on a slide dry out quickly. The rolling process is repeated until no more cellular material can be rolled onto a slide. Some slides can be left to air-dry for a Romanowsky-type stain, if preferred. Additional cellular material can still be salvaged from the brush by vortexing it in an appropriate transport or preservation medium as previously described.

The sample suspended in transport medium can be processed by cytocentrifugation or passed through a Millipore filter. More commonly, cell concentration methods are used, like the ThinPrep® Processor or SurePath PrepStain System. The advantages of these semi-automated, liquid-based preparation methods include better cell preservation, a cleaner background, and a reproducible preparation with a thin layer of cells.²³ These advantages lead to shorter screening time.

Another important advantage of the liquid-based preparations is the availability of material for ancillary studies. There is usually residual material in the transport medium for the preparation of additional slides that can be submitted for ancillary studies (e.g., immunostains) if necessary. The disadvantages include their higher cost, including the expense of the equipment and reagents, and the labor involved in preparing slides. In addition, there is a transition period for obtaining expertise in the interpretation of liquid-based preparations.²³

ACCURACY

The sensitivity and specificity of brushing cytology for the detection of malignancies of the GI tract vary according to the location and sampling method. The sensitivity of brushings for the diagnosis of GI adenocarcinomas ranges from 77% to 94%, and the specificity is generally greater than 95%.²⁴⁻³⁵ These figures are comparable to those for biopsies. The combined use of biopsy and cytology yields the highest detection rate (Table 7.1).^{7,27,30,35} The accuracy of brushing cytology is significantly higher when the brushing is performed before rather than after biopsy.^{36,37}

TABLE 7.1 DIAGNOSTIC SENSITIVITY OF ENDOSCOPIC BRUSHING AND BIOPSY IN DETECTING UPPER GASTROINTESTINAL TUMORS

Authors	Diagnostic Sensitivity (%)		
	Cytology Alone	Histology Alone	Combined
Behmard et al., 1978 ^a	92	86	—
Cook et al., 1988 ^b	85	86	91
Kobayashi and Kasugai 1978 ^c	84	77	88
Lan 1990 ^d	94	91	98
O'Donoghue et al., 1995 ^e	88	79	98
Prolla et al., 1971 ^f	91	81	100
Qizilbash et al., 1980 ^g	89	93	95
Shanghai Group 1982 ^h	77	74	88
Witzel et al., 1976 ⁱ	85	83	96
Young and Hughes 1980 ^j	92	69	—

^aBehmard S, Sadeghi A, Bagheri SA: Diagnostic accuracy of endoscopy with brushing cytology and biopsy in upper gastrointestinal lesions. *Acta Cytol* 1978;22(3):153-154.

^bCook IJ, de Carle DJ, Haneman B, et al.: The role of brushing cytology in the diagnosis of gastric malignancy. *Acta Cytol* 1988;32(4):461-464.

^cKobayashi S, Kasugai T: Brushing cytology for the diagnosis of gastric cancer involving the cardia or the lower esophagus. *Acta Cytol* 1978;22(3):155-157.

^dLan CS: Critical evaluation of the cytodiagnosis of fibrogastroendoscopic samples obtained under direct vision. *Acta Cytol* 1990;34(2):217-220.

^eO'Donoghue JM, Horgan PG, O'Donohue MK, et al. Adjunctive endoscopic brush cytology in the detection of upper gastrointestinal malignancy. *Acta Cytol* 1995;39(1):28-34.

^fProlla JC, Yoshii Y, Kirsner JB, Xavier RG: Further experience with direct vision brushing cytology of malignant tumors of upper gastrointestinal tract histopathologic correlation with biopsy. *Acta Cytol* 1971;15(4):375-378.

^gQizilbash AH, Castelli M, Kowalski MA, Churly A: Endoscopic brush cytology and biopsy in the diagnosis of cancer of the upper gastrointestinal tract. *Acta Cytol* 1980;24(4):313-318.

^hShanghai Gastrointestinal Endoscopy Cooperative Group PsRoC: Value of biopsy and brush cytology in the diagnosis of gastric cancer. *Gut* 1982;23(9):774-776.

ⁱWitzel L, Halter F, Gretillat PA, et al.: Evaluation of specific value of endoscopic biopsies and brush cytology for malignancies of the oesophagus and stomach. *Gut* 1976;17(5):375-377.

^jYoung JA, Hughes HE: Three year trial of endoscopic cytology of the stomach and duodenum. *Gut* 1980;21(3):241-246.

Table modified with permission Wang HH, Jonasson JG, Ducatman BS: Brushing cytology of the upper gastrointestinal tract. Obsolete or not? *Acta Cytol* 1991;35(2):195-198.

Because of sampling error and the presence of confounding “pseudogoblet” cells, cytology is less accurate in identifying goblet cells (for the diagnosis of Barrett’s esophagus), with a sensitivity of 41% and specificity of 84%.⁴ The use of cytology for detecting dysplasia in Barrett’s esophagus, however, is more promising. Brush cytology detects high-grade dysplasia with high sensitivity and specificity.^{2,38-41} Nonendoscopic cytologic sampling of the esophagus for surveillance has a sensitivity of approximately 90% and a specificity of over 90% for squamous dysplasia or carcinoma and a sensitivity of 80% and specificity of 95% for high-grade glandular dysplasia or adenocarcinoma.^{2,5,6,18,41} Prospective outcome studies in China of balloon cytology in a high-risk area showed that cytology results are highly predictive of the subsequent risk of esophageal and gastric-cardia cancer.⁴²⁻⁴⁴

Some authors consider cytology an unnecessary duplication of the biopsy and argue that cytology has a higher false-positive rate (lower specificity), particularly in the setting of reactive or reparative conditions.^{26,32,45} Increased experience and strict adherence to criteria for diagnosing malignancy minimize the false-positive rate and maximize the diagnostic yield of the endoscopic procedure, with a predictive value of a positive result that approaches 100%. The predictive value of a negative result is more variable, depending both on the patient population and on the sampling technique of the endoscopist.

REVIEW OF MORPHOLOGIC FINDINGS

Features noted on low-power magnification:

- cellularity
- cellular arrangements
- background

Examination of the slides under low magnification is tremendously important. Features to note are cellularity, cellular arrangements (flat sheets, three-dimensional clusters, and isolated cells), and smear background (clean, inflammatory, or necrotic). Cells in reactive processes often exfoliate as flat cohesive sheets, whereas neoplastic cells (both benign and malignant) tend to aggregate in three-dimensional clusters as a result of their proliferative tendency. Cells from a malignant neoplasm are arranged as either tight or loose three-dimensional clusters, or as a result of the loss of cellular cohesion, as isolated cells. Whereas a clean background often indicates a benign process, a dirty, inflammatory background can indicate

either a benign or a malignant process. Ulceration, whether resulting from inflammation or malignancy, is accompanied by many inflammatory cells and cellular debris. Inflammatory cells are usually numerous and predominate in reactive or reparative conditions. When many necrotic ghost cells are present without numerous inflammatory cells, a malignancy should be suspected.

Features noted on high-power magnification:

- cytoplasmic characteristics
- nuclear details

High magnification is important for the evaluation of cellular and nuclear details. In general, a benign process is characterized by a uniformity of cellular arrangement, nuclear size and shape, and the number of nucleoli. A malignant neoplasm, on the other hand, is characterized by a haphazard cellular arrangement and marked irregularities in cell size and shape, nuclear chromatin pattern, and number of nucleoli. Lymphomas and endocrine tumors are exceptions to the rule because they are often composed of similar cells.

Special features with liquid-based preparations:

- cells appear smaller
- fewer cells in sheets or groups; more single cells
- less obvious gland formation
- tumor diathesis appears as small clumps of amorphous, acellular debris

ESOPHAGUS

Infections

Esophageal infections occur most often (but not exclusively) in patients who are immunocompromised.⁴⁶⁻⁴⁹

CYTOMORPHOLOGY OF CANDIDA INFECTION:

- pseudohyphae and yeast forms
- reactive squamous cells
- [Figure 7.1](#)

Fungal infection with *Candida* species is the most common esophageal infection. Brushings are more sensitive than biopsies in the diagnosis of esophageal candidiasis.^{1,7} The number of organisms varies from few to many. Contamination by oral *Candida* is

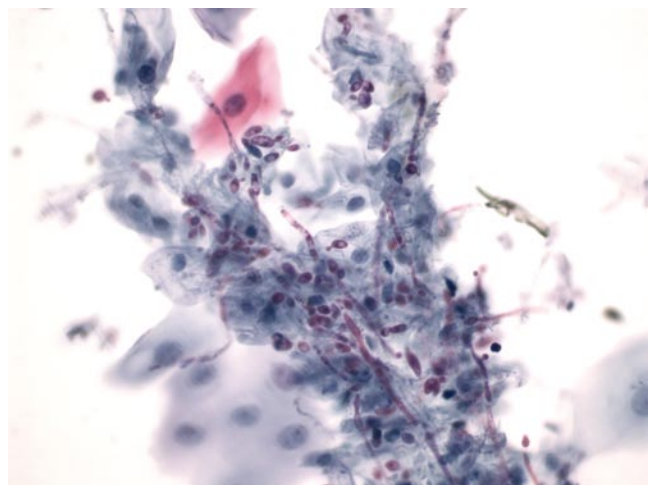


Figure 7.1 *Candida* (esophageal brushings). Infection by *Candida* species is recognized by identifying both the ovoid yeast forms and elongated pseudohyphal forms (ThinPrep, Papanicolaou stain).

not a problem because the brush is contained within a sheath, which protects it while in transit to and from the lesion.

CYTOMORPHOLOGY OF CELLS INFECTED WITH HERPES SIMPLEX VIRUS:

- multinucleation
- nuclear molding
- ground-glass chromatin
- Cowdry A bodies
- [Figure 7.2](#)

CYTOMORPHOLOGY OF CELLS INFECTED WITH CYTOMEGALOVIRUS:

- mononucleation
- large single basophilic intranuclear inclusion
- perinuclear halo
- intracytoplasmic textured inclusions

DIFFERENTIAL DIAGNOSIS OF VIRAL INFECTIONS:

- epithelial repair
- carcinoma

The large intranuclear inclusions of many viral infections can be mistaken as repair or cause confusion with malignancy.^{50,51} Molecular biologic techniques (e.g., in situ hybridization, polymerase chain reaction) can be applied to confirm the presence of virus.^{52,53} Both are equivalent to culture in their sensitivity in detecting cytomegalovirus, and both are more sensitive than morphology alone.⁵⁴

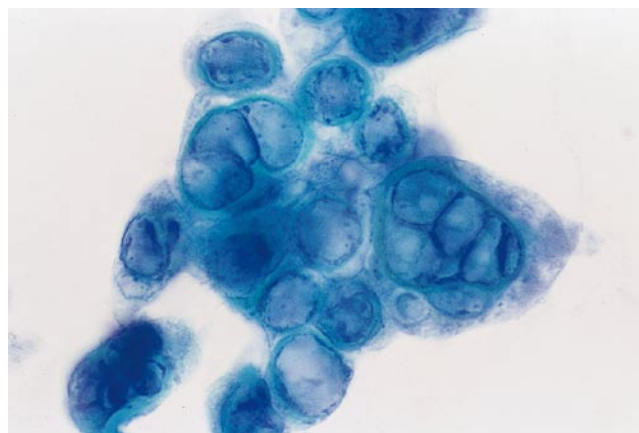


Figure 7.2 Herpes simplex infection (esophageal brushings). Multinucleation, molding of nuclei and a ground-glass chromatin pattern are diagnostic features (ThinPrep, Papanicolaou stain).

Epithelial Repair

Any injury to the mucosa, especially ulceration, evokes a cellular reaction known as epithelial repair. It is sometimes difficult to determine whether the reparative epithelium is of glandular or squamous origin.

CYTOMORPHOLOGY OF EPITHELIAL REPAIR:

- cohesive sheets with a flowing or streaming pattern noted on direct smear, but usually absent on liquid-based preparations ([Fig. 7.3](#))
- uniform nuclei with:
 - enlargement
 - smooth and thin nuclear borders
 - fine chromatin
 - prominent nucleoli
- evident mitoses
- background inflammation
- atypical stromal cells

Although epithelial repair is characterized by prominent eosinophilic nucleoli, they are usually not huge or numerous (more than three or four). The atypical stromal cells or their stripped nuclei from granulation tissue can be quite alarming. Although their nuclei are strikingly enlarged, they are not hyperchromatic; instead they have fine, homogeneous chromatin, a thin nuclear membrane, and a smooth nuclear border.

Radiation-induced changes represent a special type of reactive change or repair. There is proportional cellular and nuclear enlargement, multinucleation, metachromatic cytoplasm, and vacuolization of both cytoplasm and nuclei ([Fig. 7.4](#)).

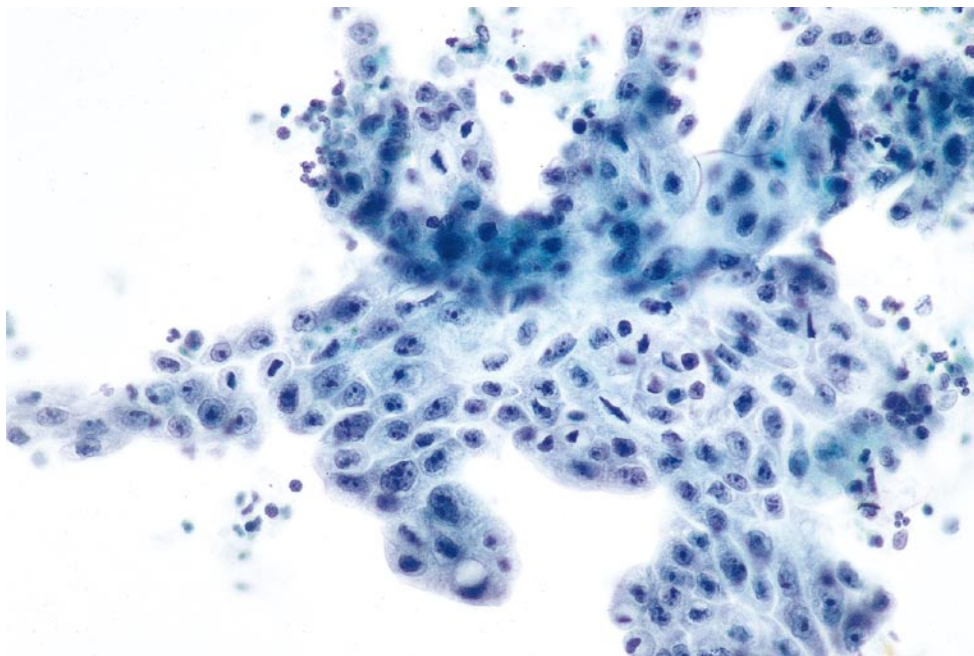


Figure 7.3 Epithelial repair (gastric brushings). A slight flowing or streaming pattern is noted, even though this is a ThinPrep preparation. The cells have slightly enlarged nuclei with regular nuclear borders, finely dispersed chromatin, and one or a few prominent nucleoli (ThinPrep, Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF REPAIR:

- dysplasia
- carcinoma

Both cellular arrangements and individual cell features are useful in this distinction. Cells with reactive or reparative changes are usually in flat sheets and there is little cell dyshesion. By contrast, neoplasms frequently show dyshesion by the “feathering” (dissociation of cells)

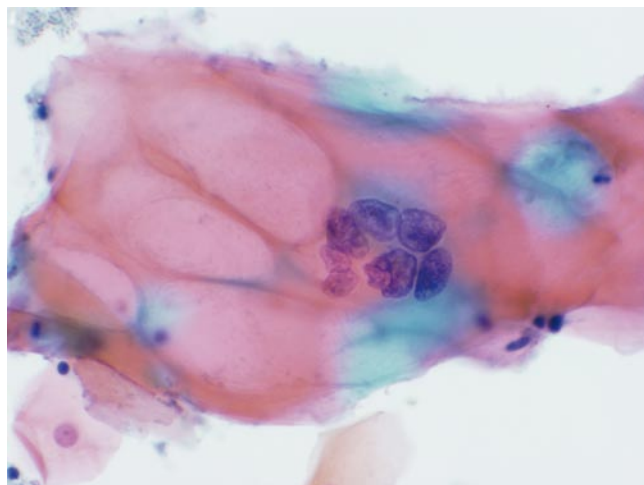


Figure 7.4 Radiation-induced changes (esophageal brushings). Cellular and nuclear enlargement, multinucleation, and vacuolization of cytoplasm and nuclei are characteristic (ThinPrep, Papanicolaou stain).

at the periphery of cell clusters or by the dispersion of numerous isolated cells. Three-dimensionality is most often associated with neoplastic lesions. In addition, although reactive or reparative cell nuclei may be enlarged, they are often uniform within the same sheet and have a similar number of regular nucleoli. In contrast, neoplastic cells, especially malignant ones, show more variation in nuclear size and shape, as well as more nuclear membrane irregularity and chromatin clumping or clearing.

Barrett's Esophagus

Barrett's esophagus (BE) is an acquired condition in which the normal stratified squamous epithelium of the distal esophagus is replaced by columnar epithelium.^{55,56} It develops as a complication in 8% to 12% of patients with chronic gastroesophageal reflux.^{56,57} According to a population-based autopsy study, the prevalence of BE is 376/100,000.⁵⁸ The vast majority (more than 90%) of esophageal adenocarcinomas arise from BE.⁵⁹⁻⁶¹ Followup studies of patients with BE show that their risk of developing esophageal adenocarcinoma is greater than the risk of lung cancer among white men aged 55 years of age or older.⁶² The columnar epithelium that replaces the squamous epithelium in the distal esophagus can be of cardiac, fundic, or intestinal type, but the increased risk of adenocarcinoma is associated with intestinal-type epithelium.⁶³⁻⁶⁵ Therefore, BE is defined by the presence of intestinal-type epithelium, characterized by goblet cells.

CYTOMORPHOLOGY OF BARRETT'S ESOPHAGUS:

- epithelial repair
- goblet cells

When obtained by brushing the esophagus, goblet cells, characterized cytologically by a single large cytoplasmic vacuole displacing the nucleus and shaping it into a crescent against the cell membrane (Fig. 7.5), are indicative of BE. Multiple goblet cells impart a Swiss cheese appearance to a honeycomb sheet of glandular cells. On liquid-based preparations, they are sometimes present as isolated cells. When goblet cells are seen on an esophageal brushing specimen, it is reasonable to report the findings as “consistent with” or “suggestive of” BE.

Significant disparities are seen between the results of brushings and biopsies in the diagnosis of BE. By this measure, cytology is neither entirely specific for nor sensitive in the diagnosis of BE.^{4,41} Much of the disparity can be accounted for by sampling. Another explanation may apply in some cases that are “diagnostic” of BE by cytology but negative by biopsy. Cells resembling goblet cells (pseudogoblet cells) have been described in the gastric cardia.^{4,66} The vacuoles of pseudogoblet cells are pink (containing neutral mucin) with hematoxylin-eosin (H & E) staining, whereas the vacuoles in goblet cells are pale blue (acid mucin). The Papanicolaou stain, however, does not distinguish between acid and neutral mucin—both are dissolved in the staining process and appear as an empty space. Alcian blue stains theoretically might help with this distinction, but they are not commonly applied to cytologic preparations.⁴ Immunocytochemical staining of cytologic specimens

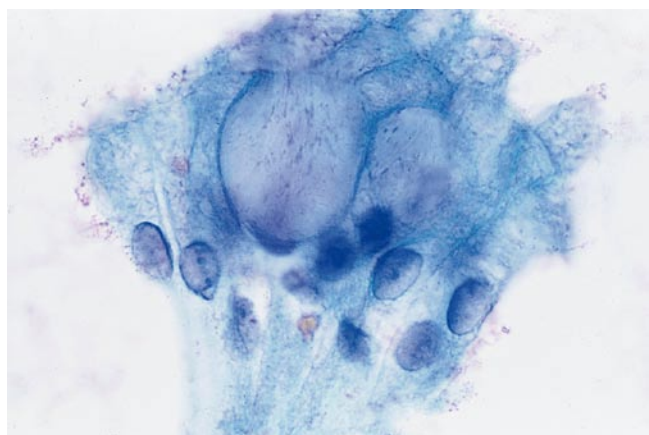


Figure 7.5 Barrett's epithelium with goblet cells. A single large cytoplasmic vacuole expands the apical portion of the cytoplasm and displaces the nucleus and shapes it into a crescent against the basal cell membrane (ThinPrep, Papanicolaou stain).

for villin and hepatocyte antigen is also helpful for identifying goblet cells on cytologic specimens but is not routine in most laboratories.^{67,68}

Dysplasia in Barrett's Esophagus

Adenocarcinomas in the setting of Barrett's epithelium arise through a sequence of mucosal alterations that include premalignant (dysplastic) esophageal epithelium.⁶⁹ Dysplastic cells have some but not all of the features of malignant cells.^{2,38,70,71}

CYTOMORPHOLOGY OF ESOPHAGEAL DYSPLASIA:

- background of Barrett's epithelium
- scattered atypical cells with some but not all features of adenocarcinoma

Preparations from an esophageal dysplasia contain fewer abnormal cells than those from an adenocarcinoma. In addition, dysplastic cells show incomplete features of malignancy (e.g., nuclear enlargement, hyperchromasia, increased nuclear-to-cytoplasmic ratio), but they generally show variation in nuclear size and shape, irregular cellular spacing, crowding, stratification, and dyshesion. The degree of these changes is proportional to the grade of dysplasia.

Although cytology is not perfect in detecting Barrett's epithelium (goblet cells), it is useful in identifying dysplasia.^{4,38,41} Grading dysplasia is clinically important because patients with low-grade dysplasia are managed with follow-up and surveillance, whereas patients with high-grade dysplasia are managed with more frequent surveillance or resection.^{56,72,73} To date, however, well-accepted criteria for diagnosing low-grade and high-grade dysplasia on cytology have not been established.⁷⁴ Based on the features described herein one may suggest that the lesion is low or high grade.^{4,41} In our practice, when a low-grade dysplasia in BE is suspected, we report the lesion as “atypical,” and describe the findings as suggestive of or consistent with low-grade dysplasia, depending on our degree of certainty. When a high-grade dysplasia is suspected, we report it as “suspicious” and conclude that the findings are suggestive of or consistent with high-grade dysplasia. We add the phrase “invasion cannot be excluded” if the cytologic findings include prominent dyshesion and many atypical cells.

CYTOMORPHOLOGY OF LOW-GRADE DYSPLASIA:

- crowded groups with stratification
- mild nuclear atypia and pleomorphism
- Figure 7.6

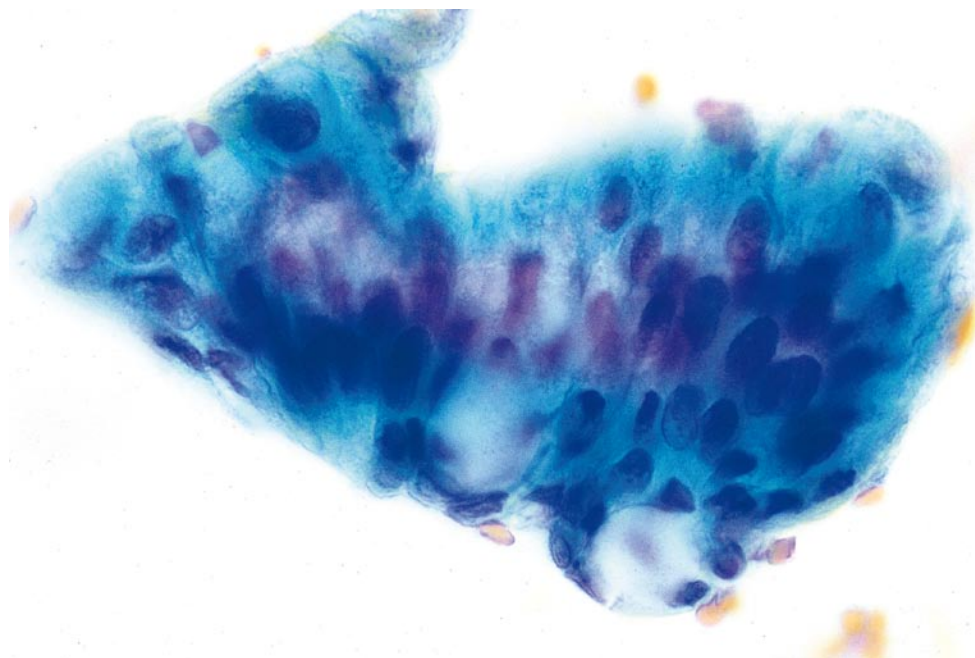


Figure 7.6 Low-grade dysplasia in Barrett's epithelium. A fragment of glandular epithelium with stratified elongated nuclei is seen. Although mucin depletion and slight nuclear enlargement are seen, significant nuclear atypia is absent (direct smear, Papanicolaou stain).

CYTOMORPHOLOGY OF HIGH-GRADE DYSPLASIA:

- crowded groups or isolated cells
- higher degree of nuclear atypia and pleomorphism
- Figure 7.7

DIFFERENTIAL DIAGNOSIS OF ESOPHAGEAL DYSPLASIA:

- regenerative epithelium
- adenocarcinoma

The diagnostic criteria for distinguishing dysplasia from reactive or reparative changes and adenocarcinoma are displayed in Table 7.2.

Dysplasia in Barrett's epithelium is often not visible endoscopically and may be missed on cytologic or histologic sampling. Thus, analysis of the molecular alterations in Barrett's epithelium may provide prognostic information as to the likelihood of progression to high-grade dysplasia or carcinoma.⁷⁵⁻⁷⁷ In a followup study by Reid and colleagues, 9 of the 13 patients with aneuploid or increased G2 or tetraploid histograms by flow cytometry developed high-grade dysplasia or adenocarcinoma. In contrast, none of the 49 patients with normal histograms progressed to high-grade dysplasia or cancer. The association of an abnormal histogram and neoplastic progression in this study was independent of the initial histologic findings. The gene and protein of p53 have also been investigated as a candidate marker for progression in the metaplasia-dysplasia-cancer sequence of BE. Although the accumulation of p53 and p21 protein

correlates with the grade, severity of dysplasia, and carcinoma on histology, its significance as a prognostic marker for progression is unclear.⁷⁸⁻⁸⁰

Adenocarcinoma of the Esophagus

Among white males in the United States, the incidence of esophageal adenocarcinoma has risen more than 350% since the mid-1970s, surpassing squamous cell carcinoma around 1990.^{60,81} Rates also rose among black males, but remained at much lower levels. Most of these tumors are located in the mid- or distal third of the esophagus, presumably arising in BE.^{61,63,82}

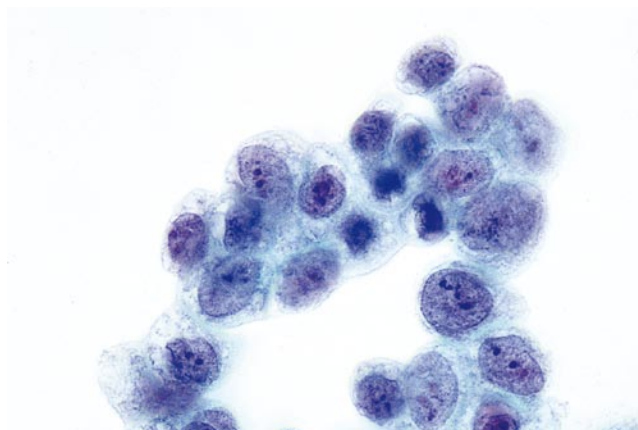


Figure 7.7 High-grade dysplasia in Barrett's epithelium. A sheet of irregularly arranged cells with variably enlarged nuclei is present without evident dyshesion. Despite the increased nuclear-to-cytoplasmic ratio, nuclear membrane irregularities, and slight hyperchromasia, the atypia is insufficient for a definitive diagnosis of malignancy (ThinPrep, Papanicolaou stain).

TABLE 7.2 COMPARISON OF CYTOLOGIC FEATURES OF REACTIVE, PREMALIGNANT, AND MALIGNANT GLANDULAR LESIONS

Cytologic Features	Reactive	Dysplasia and Adenoma	Adenocarcinoma
Tight three-dimensional clusters	Absent to rare	Absent to moderate	Moderate to many
Loose clusters	Absent to rare	Absent to moderate	Moderate to many
Dyshesion	Absent to slight	Absent to moderate	Prominent
Single atypical cells present	None to few	None to moderate	Moderate to many
Mitoses	Present	Present	Present
Atypical mitoses	None	None to few	Present
Chromatin	Vesicular	Variable	Variable
Nuclear pleomorphism	Absent to slight	Slight to moderate	Moderate to marked
Nuclear overlap	Absent to slight	Slight to moderate	Moderate to marked
Irregular nuclear spacing within tight clusters	Absent to slight	Slight to moderate	Moderate to marked
Necrosis	Absent	Absent	Occasionally present

CYTOMORPHOLOGY OF ESOPHAGEAL ADENOCARCINOMA:

- increased cellularity
- abnormal cellular arrangement
 - numerous isolated cells
 - “feathering” at the edges of cellular groups
 - haphazard crowded arrangement
 - gland formation
- atypical nuclear features (Fig. 7.8)
 - enlargement
 - hyperchromasia
 - uneven and irregular nuclear membrane
 - pleomorphism
- various amount of vacuolated cytoplasm
- tumor diathesis
- Barrett’s epithelium may or may not be present in the background

DIFFERENTIAL DIAGNOSIS OF ESOPHAGEAL ADENOCARCINOMA:

- epithelial repair
- dysplasia in Barrett’s epithelium, particularly high grade
- poorly differentiated squamous cell carcinoma

The distinction between reactive, dysplastic, and malignant esophageal lesions is challenging. Reactive or reparative cells often appear more atypical than dysplastic cells, especially those from a low-grade lesion. Whereas low-grade dysplasia consists of crowded, stratified cells with elongated nuclei, reactive cells are in sheets with a streaming pattern. Nucleoli are inconspicuous in low-grade dysplasia, whereas prominent nucleoli are characteristic of repair. High-grade dysplasia has more malignant features than low-grade dysplasia (e.g., abnormal chromatin, irregular nuclear membranes, and dyshesion), which helps to distinguish the dysplasias

from repair. The difference between a high-grade dysplasia and carcinoma is quantitative rather than qualitative: A smear from an adenocarcinoma has more abnormal cells than one from a high-grade dysplasia and the nuclear atypia in carcinoma is more marked. A tumor diathesis is a useful feature for the diagnosis of a carcinoma, but it is not present in all cases. Endoscopic findings are also important. Abnormal cells from a fungating, ulcerating lesion most likely represent carcinoma, whereas those from an indistinct flat lesion most likely represent dysplasia. It is best to report a case as “high-grade dysplasia” when a small number of atypical cells are present in a clean background from a patient undergoing surveillance for BE, particularly if endoscopy shows only granular mucosa and no definite mass. Ultimately, a biopsy is necessary for definitive diagnosis.

Squamous Cell Carcinoma of the Esophagus

Squamous cell carcinoma (SCC) is the most common malignancy of the esophagus worldwide.^{60,83} Isolated malignant cells are more commonly seen in squamous cell carcinomas, especially the well-differentiated ones, than in adenocarcinomas. Cellular features depend on the degree of differentiation.

CYTOMORPHOLOGY OF WELL-DIFFERENTIATED SQUAMOUS CELL CARCINOMA:

- hyperchromatic or pyknotic nucleus
- completely obscured chromatin
- variable cell shapes (round, oval, or spindled)
- irregular, angulated nucleus
- keratinized cytoplasm (“hard” or “glassy” orangeophilia)
- sharp cytoplasmic border
- prominent necrosis or tumor diathesis
- Figure 7.9

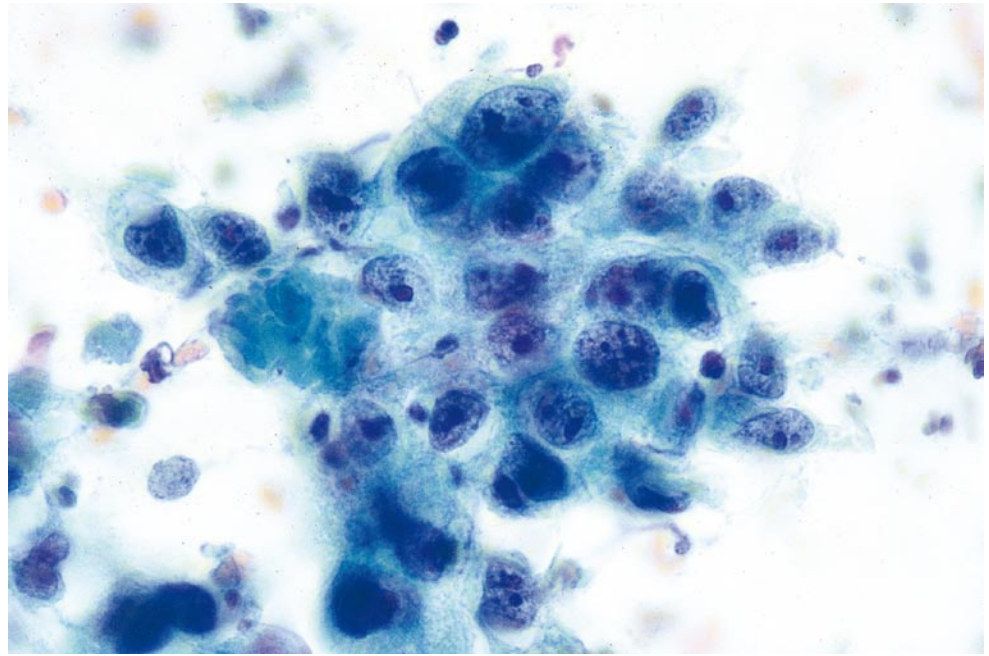


Figure 7.8 Adenocarcinoma of the esophagus. In contrast to Figure 7.7, dyshesion is evident. The nuclei show significant hyperchromasia with chromatin clumping and clearing and large prominent nucleoli (direct smear, Papanicolaou stain).



Figure 7.9 Well-differentiated squamous cell carcinoma of the esophagus. Two spindled-shaped keratinized malignant squamous cells with orangeophilic cytoplasm and hyperchromatic nuclei show markedly abnormal chromatin distribution. Degenerated cells with pyknotic nuclei are in the background (ThinPrep, Papanicolaou stain).

CYTOMORPHOLOGY OF POORLY DIFFERENTIATED SQUAMOUS CELL CARCINOMAS:

- less keratinization, nuclear angularity, pyknosis
- indistinct cell borders
- coarsely textured chromatin
- prominent nucleoli
- [Figure 7.10](#)

DIFFERENTIAL DIAGNOSIS OF SQUAMOUS CELL CARCINOMA OF THE ESOPHAGUS:

- epithelial repair
- squamous dysplasia
- poorly differentiated adenocarcinoma

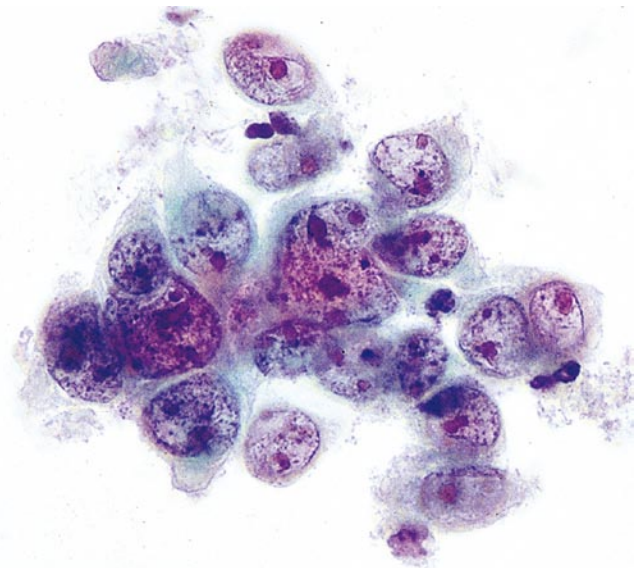


Figure 7.10 Poorly differentiated squamous cell carcinoma of the esophagus. Squamous differentiation is not discernible in this group of cells. Only scant cytoplasm is present. The nuclei show markedly abnormal chromatin distribution and prominent nucleoli (ThinPrep, Papanicolaou stain).

Squamous dysplasia of the esophagus is similar to the squamous intraepithelial lesion of the cervix. The degree of dysplasia depends on the degree of nuclear atypia, such as increased nuclear-to-cytoplasmic ratio, nuclear enlargement, hyperchromasia, coarse chromatin, and nuclear membrane irregularity. Tumor diathesis and keratinization of tumor cells are useful features to distinguish squamous dysplasia from invasive squamous cell carcinoma. Precise distinction between a poorly differentiated squamous cell carcinoma and a poorly differentiated adenocarcinoma is not always possible. Whereas adenocarcinoma cells tend to have abundant cytoplasm and eccentric nuclei, squamous cell carcinoma cells have dense cytoplasm and central nuclei. When in doubt, the tumor may be reported as non-small cell carcinoma, not further categorized.

Uncommon Tumors of the Esophagus

Histologic examination of the base of a verrucous carcinoma is crucial for correct classification, which shows a “pushing” rather than an infiltrative margin.

CYTOMORPHOLOGY OF UNCOMMON TUMORS OF THE ESOPHAGUS:

- verrucous carcinoma
 - minimal cytologic atypia
- adenosquamous carcinoma
 - both malignant squamous and glandular elements
- mucoepidermoid carcinoma
 - mucinous, squamous, and intermediate cells in varying proportions
- basaloid carcinoma
 - tight and loose groups of crowded dark basaloid cells
 - often misdiagnosed as an adenoid cystic carcinoma
- adenoid cystic carcinoma
 - cribriform, pseudoacinar, and small duct-like structures
- small cell carcinoma
 - small- or intermediate-sized cells
 - scant cytoplasm
 - prominent molding
 - necrosis and nuclear streaking common

Small cell carcinoma cells are difficult to detect on liquid-based preparations because they can mimic lymphocytes and present singly or in small groups of two or three cells. The characteristic nuclear molding is thus more difficult to recognize. In addition, more discernibly granular chromatin and occasional small nucleoli are thus seen due to better preservation.

Because leiomyomas are submucosal and usually covered by an intact mucosa, they are more often successfully sampled by endoscopic FNA rather than brushing.

The sample in the figure contains desmin-positive spindle-shaped cells (Fig. 7.11).

STOMACH

Infections

Fungal and viral infections are similar to those previously described for the esophagus. Unique to the stomach is infection by *Helicobacter pylori*. Approximately half of all adults aged 50 and older are colonized by *H. pylori*.⁸⁴ Although most are asymptomatic, their risk for developing chronic gastritis and peptic ulcers is increased. *H. pylori* infection may be a cofactor in the development of gastric carcinoma and lymphoma,⁸⁵⁻⁸⁷ but most infected patients never develop gastric cancer. The organisms can be demonstrated either on imprint smears of gastric biopsies or on brush cytology specimens.⁸⁸⁻⁹¹ Compared to histologic examination of H & E- and modified-Giemsa-stained sections, the imprint and brushing cytology are comparable in sensitivity (88%) and specificity (61%).^{88,90} The benefits of imprint and brushing cytology are rapid results, high specificity, and low cost.^{88,91} When done properly, imprint cytology does not adversely affect the quality of the biopsy specimen.⁹² With the Papanicolaou stain, *H. pylori* is a faintly basophilic, S-shaped rod admixed with mucus in the vicinity of glandular cell clusters (Fig. 7.12).^{91,93} A triple stain, combining silver, H & E, and alcian blue at acid-base balance (pH) 2.5 has a higher yield for *H. pylori* than the Papanicolaou stain.⁹⁴

Atypical mycobacteria have a characteristic appearance on cytologic preparations. Because they accumulate within macrophages, the presence of many isolated foamy histiocytes (Fig. 7.13) should raise the suspicion of an atypical mycobacterial infection. On Romanowsky-stained smears, the intracytoplasmic mycobacteria form numerous rod-shaped negative images within the histiocytes.⁹⁵ Special stains for acid-fast bacilli are necessary to confirm the diagnosis. In general, the burden of organisms is high, and they are easily seen on the acid-fast stain. Careful screening is necessary when the stain appears negative, and there is a high index of suspicion on clinical or cytologic grounds.

Epithelial Repair

Morphologically identical to its twin in the esophagus, repair in the stomach is a reactive epithelial response to gastritis and ulceration.

Dysplasia and Gastric Adenomas

Gastric dysplasia and gastric adenomas have a similar cytologic appearance, and both are precursor lesions to carcinoma. The lesion is termed *dysplasia* when it is flat and *adenoma* when polypoid. Gastric dysplasia

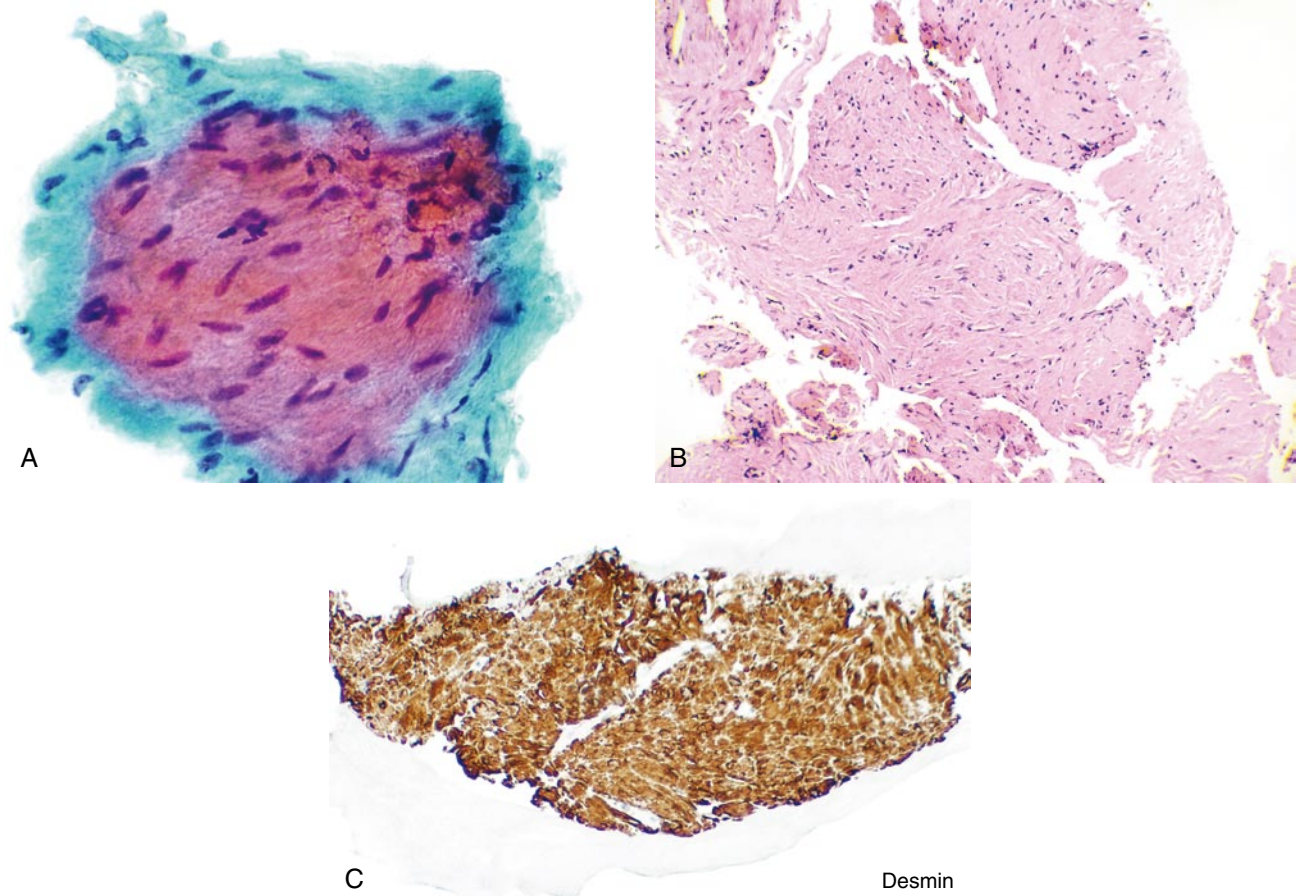


Figure 7.11 Leiomyoma of the esophagus. A, Spindle-shaped cells have abundant wavy (fibrillar) cytoplasm (ThinPrep, Papanicolaou stain). B, Cell block sections show a sparsely cellular tumor with abundant eosinophilic cytoplasm (H&E stain). C, Leiomyomas are strongly and diffusely immunoreactive for desmin.

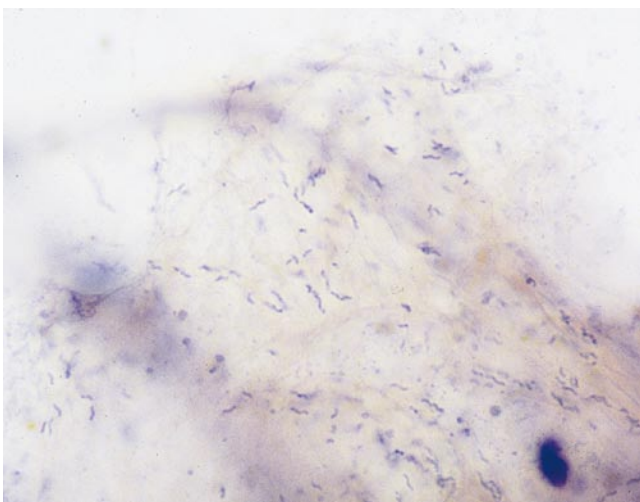


Figure 7.12 *Helicobacter pylori* (gastric brushings). Numerous faintly basophilic S-shaped rods are entrapped in mucus (direct smear, Papanicolaou stain).

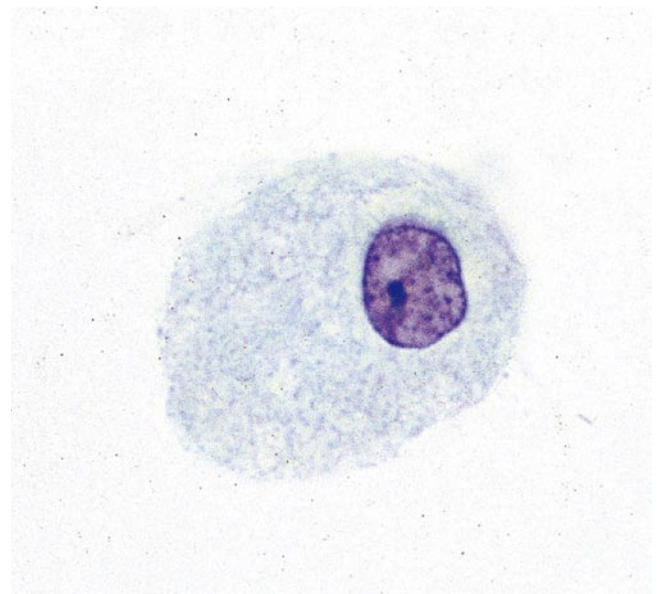


Figure 7.13 Atypical mycobacteria (duodenal brushings). An isolated histiocyte with abundant foamy cytoplasm is present. The presence of numerous acid-fast bacilli was confirmed with the acid-fast stain (direct smear, Papanicolaou stain).

is associated with atrophic gastritis and is rare in the United States. Adenomas of the stomach are also rare, unlike adenomas of the colon.

CYTOMORPHOLOGY OF GASTRIC DYSPLASIA AND ADENOMA:

- cohesive three-dimensional clusters
- uniformly enlarged nucleus
- increased nuclear-to-cytoplasmic ratio
- crowded but regular nuclear spacing
- absent or inconspicuous nucleoli

As with dysplasia in Barrett's epithelium, the cytologic appearance of gastric dysplasia and adenoma depends on the degree of dysplasia.

DIFFERENTIAL DIAGNOSIS OF GASTRIC DYSPLASIA AND ADENOMA:

- epithelial repair
- carcinoma

Crowding, a three-dimensional arrangement, and the absence of large eosinophilic nucleoli are typical of dysplasia or adenomas and help distinguish them from reparative epithelium. Cohesive groups with a regular arrangement of cells and without marked nuclear atypia or pleomorphism are typical of dysplasia or adenomas and allow distinction from a carcinoma. Some dyshesion, irregular cellular arrangement, and nuclear atypia, however, are present in high-grade dysplasia with or without a polypoid lesion. It is common for dysplasia and adenoma to coexist with adenocarcinoma. The difference between the two is often quantitative rather than qualitative and may be difficult to establish with certainty. The distinction between dysplasia and adenoma, dysplasia and adenoma with coexistent carcinoma, and carcinoma is discussed later in the chapter. Other gastric polyps, such as the hyperplastic polyp, inflammatory fibroid polyp, and fundic gland polyp, do not have distinctive cytologic features.

Adenocarcinoma of the Stomach

Gastric adenocarcinomas account for 90% to 95% of gastric malignancies and are commonly divided into two types, intestinal and diffuse (signet ring cell type). Persistent symptoms unresponsive to treatment for peptic ulcer disease, and thickened folds on radiologic or endoscopic evaluation are suspicious for a diffuse type adenocarcinoma. Because the malignant cells infiltrate predominantly the lamina propria, they are difficult to sample with a brush unless ulceration is present.

The intestinal type of gastric cancer resembles the usual types of esophageal and colorectal adenocarcinoma.

DIFFERENTIAL DIAGNOSIS OF GASTRIC ADENOCARCINOMA, INTESTINAL TYPE:

- gastric dysplasia and gastric adenoma

The most significant difference between dysplasia and the intestinal type of adenocarcinoma is cellularity and dyshesion. A smear from an adenocarcinoma is usually highly cellular, with many isolated cells and tight and loose clusters of cells in a dirty background. The evidence of malignancy is also seen in the arrangement of cells in groups and in the cytologic features of each cell, as described for esophageal adenocarcinoma.

The diffuse type of gastric cancer is composed predominantly of signet ring cells.

CYTOMORPHOLOGY OF GASTRIC ADENOCARCINOMA, SIGNET RING CELL TYPE:

- small groups or isolated cells
- vacuolated cytoplasm, often a single large vacuole
- crescent-shaped, angulated, hyperchromatic nucleus
- [Figure 7.14](#)

Signet ring cell adenocarcinoma can be quite difficult to detect on cytologic and histologic preparations. Inflammatory cells often obscure the scattered, isolated neoplastic cells. Reactive or reparative epithelial cells associated with ulceration can distract attention from the real lesion. Some signet ring cells have such a bland nucleus that they are confused with histiocytes. A high degree of suspicion is the best safeguard against failure to detect a signet ring cell carcinoma.

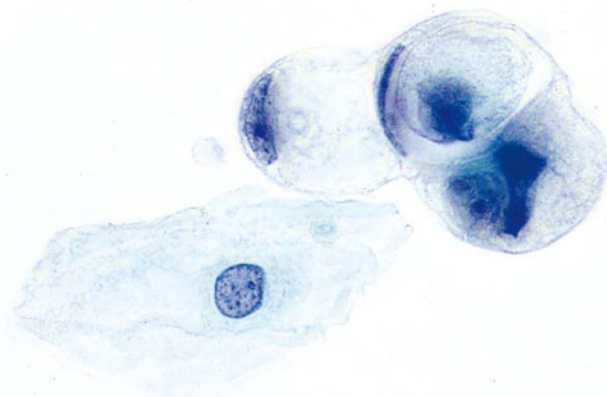


Figure 7.14 Signet ring cell carcinoma (gastric brushings). A group of malignant signet ring cells is seen. They have characteristic large vacuoles that shape the nucleus into a crescent against the cell membrane. In contrast to benign goblet cells, the nuclei in malignant signet ring cells are hyperchromatic and angulated (ThinPrep, Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF GASTRIC ADENOCARCINOMA, SIGNET RING CELL TYPE:

- inflammation (macrophages)
- intestinal metaplasia

Cells of signet ring adenocarcinoma are distinguished from histiocytes because adenocarcinoma cells lack phagocytosis and generally have some nuclear hyperchromasia and pleomorphism. A careful search for clusters of atypical cells helps to confirm the epithelial nature of the cells: histiocytes generally do not show cellular cohesion. When in doubt, immunostains can be used to determine the epithelial versus histiocytic nature of signet ring type cells. Epithelial signet ring cells are positive for epithelial markers (e.g., keratin, EMA). Histiocytes are negative for these markers but express CD68. Goblet cells from intestinal metaplasia are rarely present as single cells, except in liquid-based preparations. They do not show nuclear atypia like angulation and hyperchromasia.

Endocrine Tumors (Including Carcinoid Tumor)

Endocrine tumors, including carcinoid tumors, are derived from the diffuse endocrine system of the GI tract, which is composed of amine- and peptide-producing cells.^{96,97} GI endocrine tumors are classified into three major categories that include 1) well-differentiated endocrine tumors, 2) well-differentiated endocrine carcinomas, and 3) poorly differentiated endocrine (small cell) carcinomas.⁹⁸ The term *carcinoid tumor* encompasses tumors in the first two categories.⁹⁹ The distinction between these two categories is based on size and site, the presence of local invasion, angio invasion, and metastases. Cytologic atypia, mitotic index, proliferative rate as assessed by MIB-1, and patterns of hormone production are additional important parameters of the classification. The GI tract is the most common site for carcinoid tumors that have a low malignant potential.¹⁰⁰ The small intestine is the most common site for endocrine tumors of the GI tract, followed by the rectum and appendix; the stomach is a distant fourth.¹⁰⁰ They account for less than 1% of all gastric malignancies.^{83,101} Cytologic specimens are rarely obtained from the ileum, rectum, or appendix.

CYTOMORPHOLOGY OF ENDOCRINE TUMORS:

- dyshesive monomorphic cells
- plasmacytoid
 - eccentric, round to oval nucleus
 - moderate amount of basophilic, dense cytoplasm
 - no perinuclear clear zone ("hof")

- many stripped nuclei
- finely granular ("salt-and-pepper") chromatin
- Figure 7.15

Poorly differentiated endocrine tumors have a more variable appearance.

DIFFERENTIAL DIAGNOSIS OF ENDOCRINE TUMORS:

- adenocarcinoma
- small cell carcinoma
- non-Hodgkin lymphoma

Endocrine tumors may be confused with a small cell lymphoma because the cytologic smears of endocrine tumors occasionally show isolated, monomorphic cells or even bare nuclei. One way to avoid this pitfall is to identify intact cells and base a diagnosis on these cells only. Intact cells of endocrine tumors have more abundant cytoplasm than those of a small cell lymphoma.

It can be difficult to distinguish a poorly differentiated endocrine tumor from an adenocarcinoma. Nuclear monomorphism and finely granular chromatin raise the suspicion of endocrine differentiation, which can be confirmed by immunocytochemical studies.

The distinction between a poorly differentiated endocrine tumor (small cell carcinoma) and a lymphoma can be difficult; nuclear molding and a finely dispersed chromatin pattern are features that favor small cell carcinoma. Immunocytochemical stains for keratin, chromogranin, and LCA are helpful. Endocrine tumors are invariably positive for keratin and negative for LCA. Chromogranin is often expressed in a well-differentiated endocrine tumor, and may be absent in a poorly differentiated one.

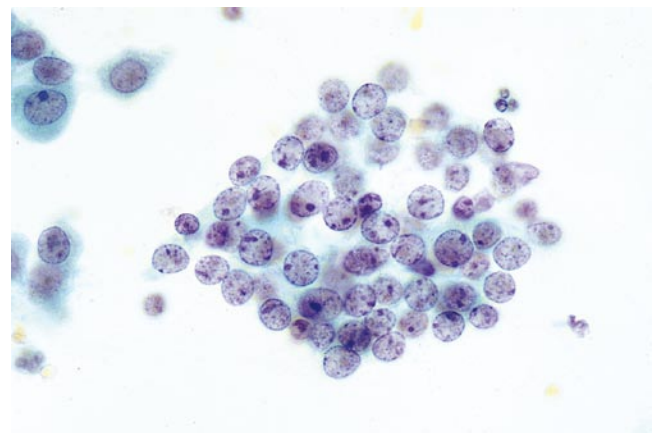


Figure 7.15 Well-differentiated endocrine (carcinoid) tumor (duodenal brushings). A group of dyshesive monomorphic stripped nuclei is present in the center. A salt and pepper chromatin pattern is evident. A few intact cells with a plasmacytoid appearance are seen at the edge (direct smear, Papanicolaou stain).

TABLE 7.3 COMPARISON OF CYTOLOGIC AND IMMUNOCYTOCHEMICAL FEATURES OF LYMPHOMA, CARCINOMA, AND ENDOCRINE TUMOR

	Large Cell Lymphoma	Small Cell Lymphoma	Carcinoma	Endocrine Tumor
Arrangement of cells	Isolated and scattered with occasional clusters, depending on cellularity	Same as large cell lymphoma	Isolated and groups with irregular nuclear spacing and superimposition	Isolated and loose clusters
Cytoplasmic vacuolization	Small vacuoles, if present	Quite rare	Irregular vacuolization	Small vacuoles, if present
Phagocytosis	Rare	Not observed	Rare	Not observed
Chromatin pattern	Vesicular	Slightly coarse	Variable with chromatin clumping	Finely to somewhat coarsely granular
Nuclear monomorphism	Present	Present	Absent	Present
Nucleolus	Prominent, enlarged, or small multiple	Not prominent	Can be prominent, enlarged	Generally not prominent
Immunocytochemistry				
Keratin	–	–	+	+
Epithelial membrane antigen (EMA)	–	–	+	–
Leukocyte common antigen (LCA)	+	+	–	–
Chromogranin	–	–	–	+

A comparison of the cytologic and immunocytochemical features of lymphoma, carcinoma, and endocrine tumor is provided in [Table 7.3](#).

Non-Hodgkin Lymphoma

Non-Hodgkin lymphoma is the second most common malignancy of the stomach, accounting for approximately 5% of all gastric malignancies, and its incidence is increasing.^{83,102} The stomach is, in fact, the most common site for extranodal non-Hodgkin lymphomas.¹⁰³ In 1983, the histologic relationship of extranodal B-cell lymphomas and mucosa-associated lymphoid tissue (MALT) was recognized, and the MALT lymphoma concept was proposed.^{87,104}

By definition, MALT lymphomas are small-cell, low-grade lymphomas. The existence of a “large-cell, high-grade MALT lymphoma” is controversial. Although some investigators believe that MALT lymphomas may undergo high-grade transformation,¹⁰⁵⁻¹⁰⁷ most agree to restrict the term *MALT lymphoma* to low-grade B-cell lymphomas. In doing so, one avoids implying that “high-grade” MALT lymphomas may respond to antiobiotic therapy, as low-grade MALT lymphomas often do.¹⁰⁸ As a result, MALT lymphoma is characterized by a diffuse infiltrate of small- to medium-sized lymphoid cells of B-cell phenotype. A central feature of MALT lymphoma is the lymphoepithelial lesion, an epithelial structure infiltrated by neoplastic B cells.

The most common non-Hodgkin lymphoma of the stomach, albeit less thoroughly investigated than MALT lymphoma, is the diffuse large B-cell lymphoma

(DLBL), which constitutes 55% to 65% of all gastric lymphomas.¹⁰⁹⁻¹¹¹ DLBL consists of large lymphoid cells and the lymphoepithelial lesions are not as frequent as in MALT lymphoma.¹⁰⁴ Primary gastric non-Hodgkin lymphoma needs to be differentiated from secondary involvement of the stomach by a nodal lymphoma. On gastric brushings, the challenge is to diagnose the atypical cells as lymphoid and to broadly classify them as “large cell” or “small cell.” The final subtyping depends on clinical correlation and larger tissue sampling for morphologic and immunophenotypic or molecular studies.

The cellularity and the background elements of a gastric brushing specimen from a non-Hodgkin lymphoma vary depending on the presence or absence of ulceration. Because the tumor cells are predominantly in the lamina propria, cellularity is high only when ulceration exposes them to the brush. Otherwise, if the mucosa is intact, diagnostic cells may be few or absent altogether. As a result, only two thirds or fewer of biopsy-proven gastric lymphomas are detected on cytologic preparations.^{8,112,113} Ulceration also gives the smear a dirty background, with many reactive or reparative epithelial cells. Endoscopic FNA, particularly under ultrasound guidance, may increase the diagnostic yield.^{17,114,115}

CYTOMORPHOLOGY OF NON-HODGKIN LYMPHOMA:

- numerous isolated cells
- scant cytoplasm

The cytologic appearance varies according to the subtype of lymphoma. The large cell type does not usually pose any diagnostic difficulty because the cells have large atypical nuclei that have a single large nucleolus or multiple small but prominent nucleoli (Fig. 7.16).^{8,116}

DIFFERENTIAL DIAGNOSIS OF LARGE CELL LYMPHOMA:

- poorly differentiated carcinoma

It is often easier to recognize malignant lymphoid features on a well-prepared cytologic smear than on a biopsy, which tends to suffer from crush artifact. Small cell lymphomas (Fig. 7.17) are more difficult to diagnose and may be confused with an inflammatory condition.

DIFFERENTIAL DIAGNOSIS OF SMALL CELL LYMPHOMA:

- chronic inflammation
- endocrine tumor

The absence of any true cellular cohesion, as would be expected in poorly differentiated carcinoma, is the principal diagnostic feature of a lymphoma. In addition, a poorly differentiated carcinoma often has more abundant and vacuolated cytoplasm and a greater degree of nuclear pleomorphism than a large cell lymphoma. Although a poorly differentiated carcinoma may sometimes present a pattern of isolated cells or bare nuclei, a careful search of all the available material will usually uncover small groups or fragments of malignant cells that are recognizably epithelial. Immunocytochemical staining may facilitate the distinction. Carcinomas are positive for keratin and EMA, and negative for LCA.

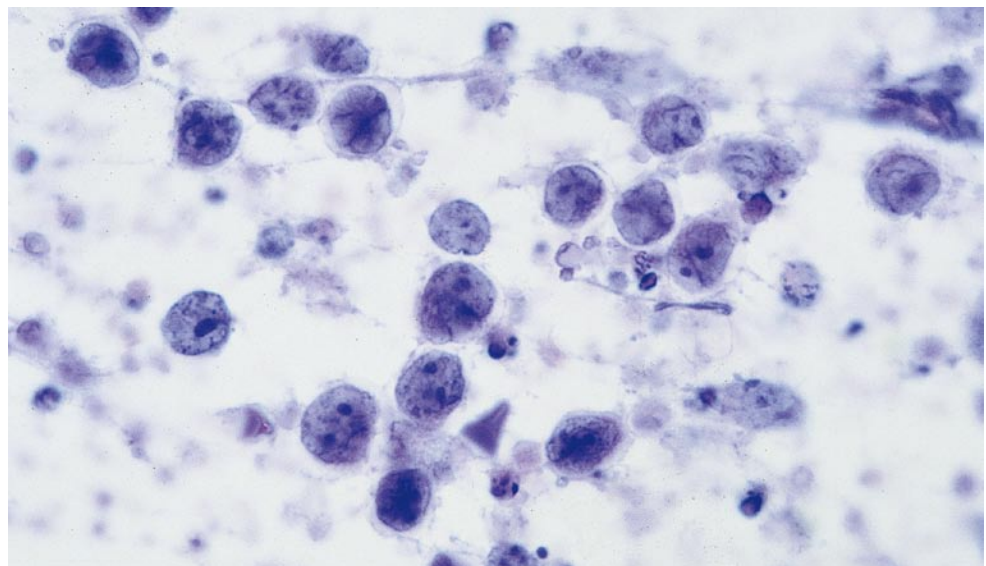
Lymphomas, on the other hand, are negative for keratin and EMA and positive for LCA. Because the majority of lymphomas in the GI tract are B-cell lymphomas, they also show positive staining for CD20, a marker for B cells. A CD30-positive anaplastic large cell lymphoma, however, may be positive for EMA while negative for LCA.⁸

A small cell lymphoma, whether MALT or follicular lymphoma, can be mistaken for chronic inflammation on cytologic smears. A predominance of lymphoid cells over neutrophils in an apparently inflammatory smear should raise the suspicion of a lymphoma. The presence of many monomorphic, atypical, small, or small and large lymphoid cells strongly suggests a small cell or a mixed lymphoma. Marker studies are almost always required to confirm the diagnosis. A cytologic diagnosis suggesting a small cell lymphoma should be confirmed whenever possible by tissue examination. Cells of an endocrine tumor generally have more cytoplasm than the cells of a small cell lymphoma; their nuclei are round and have a more finely granular chromatin than that of lymphoid cells.

Gastrointestinal Stromal Tumor

Virtually all mesenchymal tumors of the GI tract were once considered to be smooth muscle tumors. A subgroup has been redefined as GI stromal tumors (GISTs), based in large part on their immunoreactivity for c-kit (CD117).¹¹⁷ They recapitulate differentiation toward the interstitial cells of Cajal, which are the c-kit expressing cells that act as the peristaltic pacemakers of the GI tract.¹¹⁸ Immunoreactivity for c-kit, and to a lesser degree, CD34, distinguishes GISTs from true smooth muscle tumors, which are positive for desmin and smooth muscle actin but negative for c-kit. Positive staining for antibodies to c-kit (CD117) is now virtually essential for their diagnosis.¹¹⁹

Figure 7.16 Diffuse large B-cell lymphoma (DLBL; endoscopic fine-needle aspiration [FNA] of a gastric mass). Scattered isolated large atypical cells with scant cytoplasm are seen. The nuclei have vesicular chromatin with one or a few prominent nucleoli. Necrotic debris and apoptotic bodies are present in the background (direct smear, toluidine blue and eosin stain).



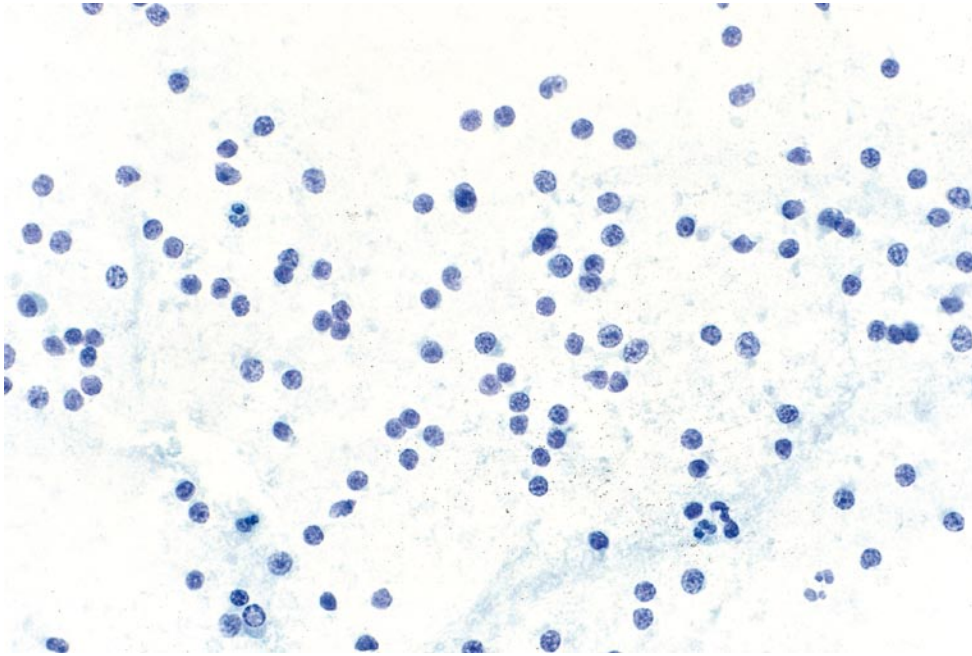


Figure 7.17 Lymphoma of mucosa-associated lymphoid tissue (MALT) type (gastric brushings). Scattered lymphocytes are seen without intermixed neutrophils or plasma cells (direct smear, Papanicolaou stain).

GISTs are classified as spindled (70%) or epithelioid (30%), depending on their cell composition. Most are benign, but 10% to 30% are malignant, with characteristic intra-abdominal dissemination and metastases to the liver.

CYTOMORPHOLOGY OF GASTROINTESTINAL STROMAL TUMOR:

- irregularly outlined clusters of cells
- spindle-shaped or epithelioid
- wispy cytoplasm with long extensions
- mitoses, necrosis (occasionally)

Because of its submucosal or mural location, a GIST is usually not accessible to the endoscopic brush unless the overlying mucosa is ulcerated. FNA is the preferred method of sampling.^{17,120} The cytologic appearance depends on the cell type. The specimens are usually cellular, with isolated cells and loose and crowded fragments of spindle or epithelioid cells (Fig. 7.18). The individual cells often lose their wispy cytoplasm and become stripped nuclei.¹²¹⁻¹²³ Perinuclear or paranuclear vacuoles are present in some cells.¹²¹ Delicate cytoplasm and prominent nuclear palisading have also been noted.^{122,124} Analysis of kit gene by

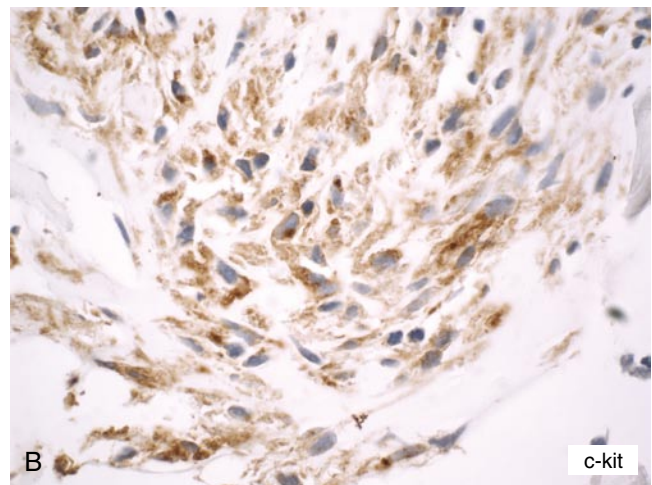
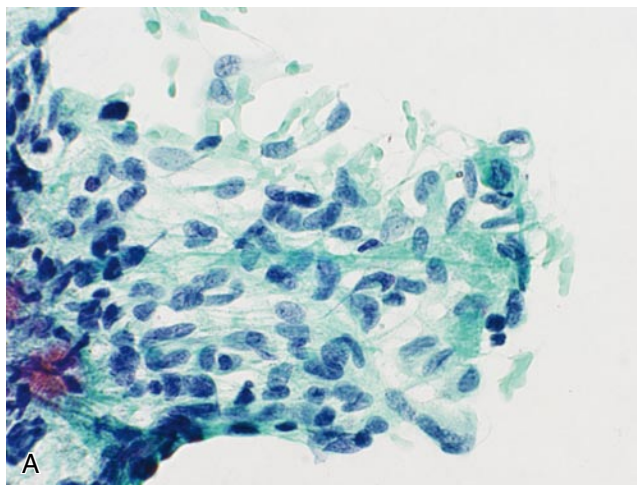


Figure 7.18 Gastrointestinal stromal tumor (ultrasound-guided endoscopic fine-needle aspiration [FNA] of a gastric mass). A, The cells have an oval nucleus with bland chromatin and an inconspicuous nucleolus (direct smear, Papanicolaou stain). B, The corresponding cells in a cell block preparation stain positively for c-kit in a characteristic dot-like cytoplasmic pattern.

polymerase chain reaction and immunocytochemical staining for CD117 can be applied to the aspirated material to confirm the diagnosis.^{122,125} The distinction between a benign and malignant GIST is best reserved for gross and histologic examination of the resected specimen.

DIFFERENTIAL DIAGNOSIS OF GASTROINTESTINAL STROMAL TUMOR:

- leiomyoma
- leiomyosarcoma

Leiomyomas of the GI tract usually involve the esophagus (see Fig. 7.11) and colorectum. They are less common in the stomach. Endoscopic FNAs yield spindle-shaped cells. Leiomyosarcomas tend to show more significant nuclear pleomorphism, nuclear atypia, and mitoses, and a less prominent vascular pattern than GIST. The definitive distinction between the smooth muscle tumors and GIST depends on differential immunocytochemical expression of CD117 (c-kit), CD34, and desmin.¹¹⁹

DUODENUM

Infections

Brush cytology of the duodenum is useful to detect *Giardia lamblia*, which is an intestinal parasite that is usually acquired from contaminated drinking water and may cause diarrhea.^{126,127} The organism is flat, gray, pear-shaped, and binucleate, with four pairs of flagella.

Intestinal infection with *Microsporidium*, an obligate intracellular spore-forming protozoon, occurs as an opportunistic infection in patients with acquired immune deficiency syndrome (AIDS). Microsporidia can be detected on cytologic specimens (e.g., urine, stool, nasal secretions, duodenal aspirates, bile, and brushings from the duodenum and biliary tract).¹²⁸⁻¹³⁰ With the Papanicolaou stain they appear in aggregates as brightly eosinophilic rod-shaped or ovoid organisms measuring 1 to 3 μm in diameter (Fig. 7.19). When intracellular, they are in the supranuclear portion of the cytoplasm. They may be missed for two reasons: 1) the pathologist may be distracted by the reactive changes as a result of the infection, and 2) they are at a slightly different plane of focus from that of the nucleus. It is helpful to keep this

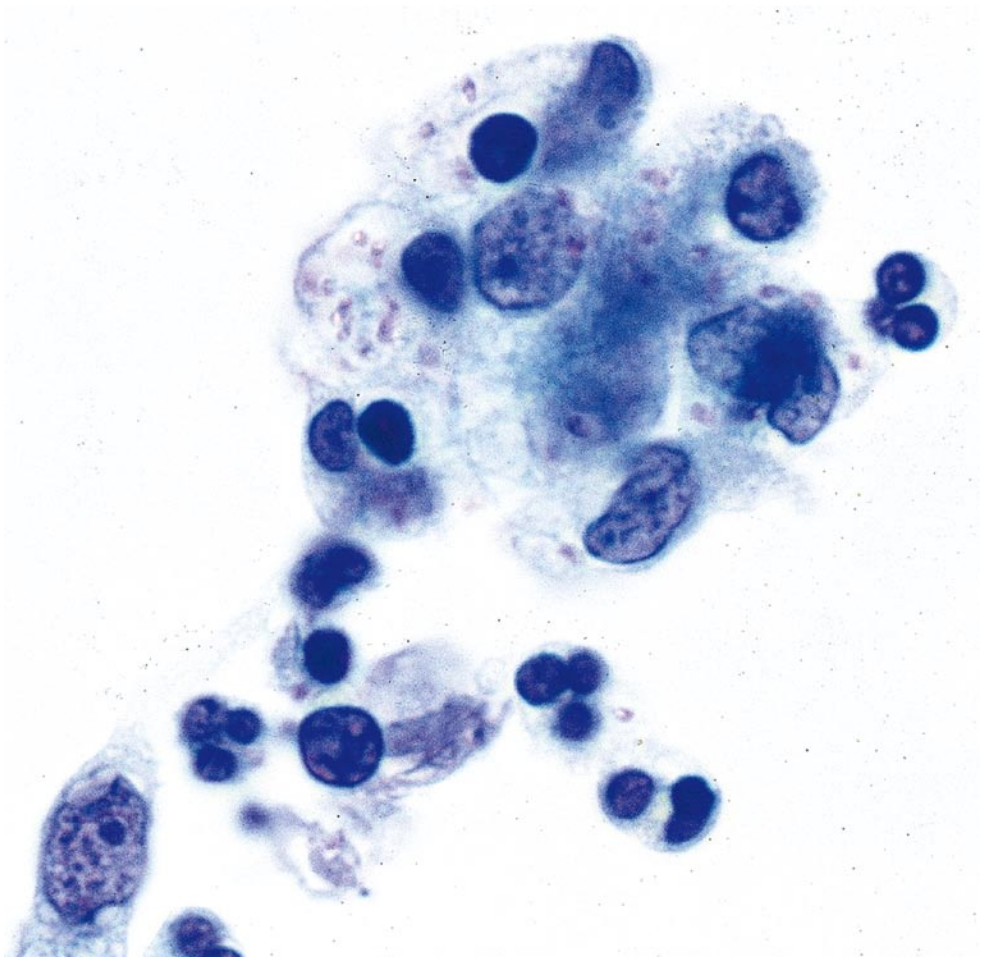


Figure 7.19 Microsporidia. Aggregates of brightly eosinophilic rod-shaped or ovoid organisms, measuring 1 to 3 μm in diameter, are seen in the apical portion of glandular epithelial cells (ThinPrep, Papanicolaou stain).

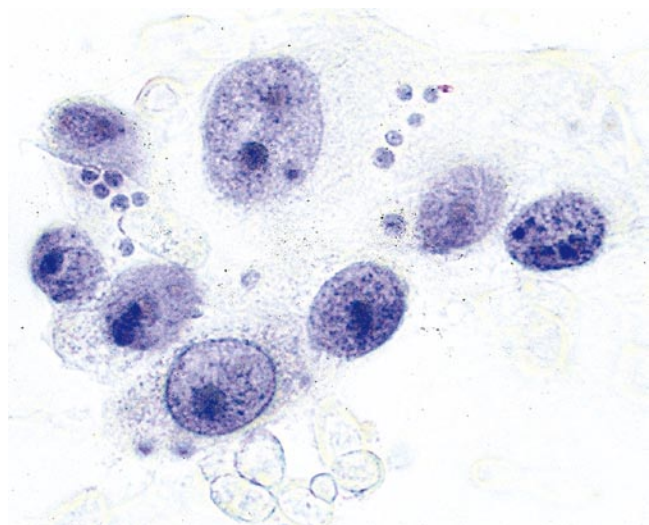


Figure 7.20 Cryptosporidia (gastric brushings). Aggregates of 2- to 5- μ m diameter round basophilic bodies are seen on the surface of glandular epithelial cells (direct smear, Papanicolaou stain).

possibility in mind when one examines a specimen from a patient who is immunocompromised. Their presence can be confirmed with the Brown-Brenn stain.

Cryptosporidia can involve any glandular epithelium (stomach, bile duct, small and large intestine) of the GI tract in patients with human immunodeficiency virus (HIV) infection.¹³¹ Like microsporidia, Cryptosporidia can be detected by light-microscopic examination of stool and other cytology specimens. Cryptosporidia are 2- to 5- μ m diameter round basophilic bodies (Fig. 7.20) on the luminal surface of the epithelial cells. They are seen only when the plane of focus is shifted to the surface of the cells where the organisms reside. When in doubt, a confirmatory Grocott methenamine silver stain can be applied.

Other common lesions of the duodenum, including epithelial repair, adenocarcinomas, endocrine tumors, and infections, are similar to their counterparts in the stomach.

Adenoma and Adenocarcinoma

Premalignant lesions, including adenomas, are more common in the colon than in the stomach or small bowel. The majority (60% to 70%) of adenomas in the duodenum are associated with an adenocarcinoma.¹³²⁻¹³⁴ Adenomas of the duodenum resemble those found in the stomach and colon (Fig. 7.21).

CYTOMORPHOLOGY OF DUODENAL ADENOMA:

- cohesive three-dimensional clusters of crowded epithelial cells
- increased nuclear-to-cytoplasmic ratio
- absent goblet cells
- palisading and molding of elongated nuclei
- fine chromatin and absent or small nucleoli

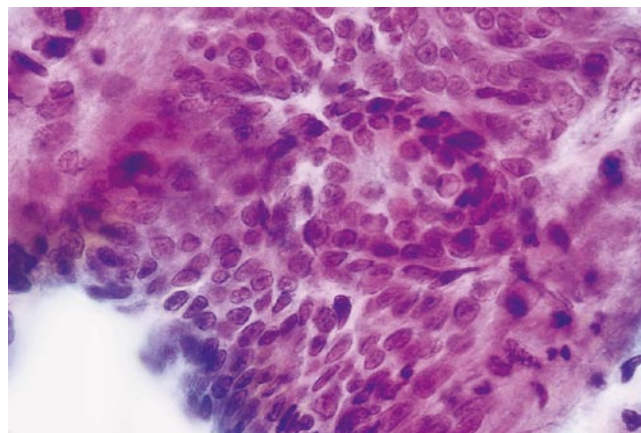


Figure 7.21 Ampullary adenoma (ampullary brushings). A crowded group of glandular cells with mucin depletion and an increased nuclear-to-cytoplasmic ratio is present. A gland opening is apparent. Despite the crowding, the arrangement is orderly. The nuclei are enlarged and elongated but significant atypia is absent (ThinPrep, Papanicolaou stain).

When we are certain that the lesion represents an adenoma, we report it as “positive for neoplastic cells, consistent with adenoma”; otherwise, we report it as “atypical glandular cells present, suggestive of an adenoma.” A duodenal adenoma may coexist with adenocarcinoma. It is easier to distinguish a low-grade premalignant lesion from a carcinoma; a high-grade premalignant lesion may be quite difficult to distinguish from a malignant one.¹³⁵ A high-grade lesion is also more likely to coexist with a carcinoma. The degree of cellularity, dyshesion, and nuclear atypia helps to distinguish a high-grade premalignant lesion from a frankly malignant one, but the difference is quantitative, not qualitative.⁷¹ Malignant lesions show greater cellularity, more dyshesion, and more marked nuclear atypia and pleomorphism. A comparison of the cytologic features of reactive, premalignant, and malignant glandular lesions is shown in Table 7.2. Even when only a low-grade premalignant lesion is present on the cytologic specimen, an unsampled coexisting carcinoma elsewhere in the lesion cannot be excluded. Therefore, surgical resection of the entire lesion is indicated when a diagnosis of a premalignant lesion of the small bowel is suspected or rendered on cytology. When the lesion is diffuse or ill-defined, multiple biopsies should be taken.

COLON

The morphologic findings of the common lesions of the colon, including epithelial repair, adenomas (Fig. 7.22), adenocarcinomas, lymphomas, and infections, are similar to those described previously for the same lesions elsewhere in the GI tract. The cytologic findings in another common condition of the colon, ulcerative colitis, have been described by some authors; however, it is not customary to obtain cytologic specimens in this setting.¹³⁶⁻¹⁴⁰

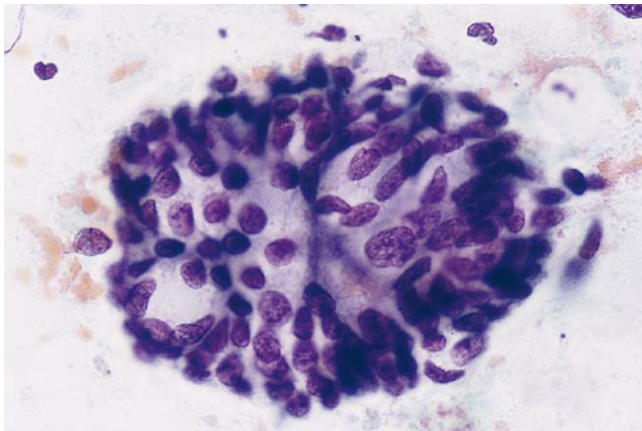


Figure 7.22 Colon adenoma (colonic brushings). A cohesive group of stratified but orderly glandular cells with elongated nuclei is seen. Despite the increased nuclear-to-cytoplasmic ratio and hyperchromasia, significant atypia is absent (direct smear, Papanicolaou stain).

Cytology of the colon is less commonly used than cytology of the upper GI tract. One reason may be that colonoscopic examinations are widely performed for colitis, particularly inflammatory bowel disease, and cytologic examination is not useful in establishing the diagnosis of active or chronic colitis.¹³⁶ The distinctions between repair, dysplasia, and carcinoma on cytologic preparations in cases of inflammatory bowel disease is difficult if not impossible. The role of brushing cytology to increase the yield of identifying dysplasia or carcinoma in this context is controversial.^{136,137} Colonic adenomas are common, and cytologic examination cannot be relied on to exclude the possibility of an invasive carcinoma in these polyps. Colonic cytology is thus limited in its capacity to correctly categorize many of the common lesions of the colon. Nonetheless, the combined use of both biopsy and cytology renders the highest detection rate for malignancy, as has been shown with lesions of the upper GI tract.¹⁴¹⁻¹⁴⁶

THE ANAL PAP TEST

Persistent human papillomavirus (HPV) infection at the ano-rectal transformation zone is in many ways analogous to HPV infection at the cervical transformation zone and is just as capable of causing cancer. Squamous cell carcinoma of the anus occurs in men and women but is an especially serious health problem in gay men. The incidence of anal squamous cell carcinoma in men who have sex with men is 35 per 100,000, roughly the same as the incidence of cervical cancer before the introduction of Pap screening.^{147,148} The estimated incidence of anorectal cancers in the United States is approximately 4,800 cases per year.¹⁴⁹

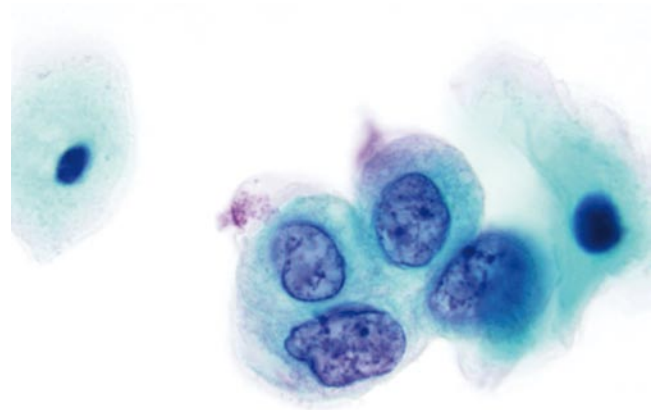


Figure 7.23 High-grade squamous intraepithelial lesion (anal Pap). Cells show nuclear enlargement, nuclear membrane irregularity, and chromatin coarsening, analogous to the changes seen in cervical specimens (ThinPrep, Papanicolaou stain).

Screening for anal cancer and its precursors was implemented in the 1990s, with performance characteristics similar to those for cervical lesions.¹⁴⁸ Sample procurement is simple and does not require an anoscope. The sample is collected with the patient in the lateral recumbent or dorsal lithotomy position. A Dacron swab moistened with tap water is inserted blindly 5 to 6 cm into the anal canal past the anal verge and into the rectal vault. The swab is rotated with firm lateral pressure and slowly withdrawn. Smears or liquid-based slide preparations are equally acceptable.

Anal squamous intraepithelial lesions and squamous cell carcinomas are morphologically identical to their counterparts in the cervix (Fig. 7.23). For this reason, Bethesda 2001 terminology (e.g., NILM, ASC-US, ASC-H, LSIL, HSIL; see Chapter 1) is commonly used for reporting results. Adequacy criteria differ slightly from those for cervical cytology. The minimum cellularity is approximately 2,000 to 3,000 nucleated squamous cells, which is equivalent to one or two nucleated squamous cells per $\times 40$ field with a ThinPrep or MonoPrep slide, or three to six nucleated squamous cells per $\times 40$ field with a SurePath slide.¹⁵⁰ The presence of squamous metaplastic cells and rectal columnar cells should be reported to indicate anal transformation zone sampling.

REFERENCES

1. Jhala N, Jhala D: Gastrointestinal tract cytology: Advancing horizons. *Adv Anat Pathol* 2003;10(5):261-277.
2. Geisinger KR, Teot LA, Richter JE: A comparative cytopathologic and histologic study of atypia, dysplasia, and adenocarcinoma in Barrett's esophagus. *Cancer* 1992;69:8-16.
3. Howell LP, Wright AL, Calafati SA, et al.: Cytodiagnosis of in situ and early carcinoma of the upper gastrointestinal tract. *Acta Cytol* 1985;29(3):269-273.
4. Wang HH, Sovie S, Zeroogian JM, et al.: Value of cytology in detecting intestinal metaplasia and associated dysplasia at the gastroesophageal junction. *Hum Pathol* 1997;28(4):465-471.

5. Falk GW, Chittajallu R, Goldblum JR, et al.: Surveillance of patients with Barrett's esophagus for dysplasia and cancer with balloon cytology [see comments]. *Gastroenterology* 1997;112(6):1787-1797.
6. Leoni-Parvex S, Mihaescu A, Pellanda A, et al.: Esophageal cytology in the follow-up of patients with treated upper aerodigestive tract malignancies. *Cancer* 2000;90(1):10-16.
7. Wang HH, Jonasson JG, Ducatman BS: Brushing cytology of the upper gastrointestinal tract. Obsolete or not? *Acta Cytol* 1991;35(2):195-198.
8. Sherman ME, Anderson C, Herman LM, et al.: Utility of gastric brushing in the diagnosis of malignant lymphoma. *Acta Cytol* 1994;38(2):169-174.
9. Ducatman BS, Hogan CL, Wang HH: A triage system for processing fine needle aspiration cytology specimens. *Acta Cytol* 1989;33(6):797-799.
10. Yang GC, Alvarez II: Ultrafast Papanicolaou stain. An alternative preparation for fine needle aspiration cytology. *Acta Cytol* 1995;39(1):55-60.
11. Iishi H, Yamamoto R, Tatsuta M, Okuda S: Evaluation of fine-needle aspiration biopsy under direct vision gastrofiberscopy in diagnosis of diffusely infiltrative carcinoma of the stomach. *Cancer* 1986;57(7):1365-1369.
12. Ingoldby CJ, Mason MK, Hall RI: Endoscopic needle aspiration cytology: A new method for the diagnosis of upper gastrointestinal cancer. *Gut* 1987;28(9):1142-1144.
13. Kochhar R, Rajwanshi A, Malik AK, et al.: Endoscopic fine needle aspiration biopsy of gastroesophageal malignancies. *Gastrointest Endosc* 1988;34(4):321-323.
14. Lange P, Kock K, Laustsen J, et al.: Endoscopic fine-needle aspiration cytology of the stomach. A new diagnostic procedure. *Endoscopy* 1987;19(2):72-73.
15. Layfield LJ, Reichman A, Weinstein WM: Endoscopically directed fine needle aspiration biopsy of gastric and esophageal lesions. *Acta Cytol* 1992;36(1):69-74.
16. Wiersema MJ, Hawes RH, Tao LC, et al.: Endoscopic ultrasonography as an adjunct to fine needle aspiration cytology of the upper and lower gastrointestinal tract. *Gastrointest Endosc* 1992;38(1):35-39.
17. Wiersema MJ, Wiersema LM, Khusro Q, et al.: Combined endosonography and fine-needle aspiration cytology in the evaluation of gastrointestinal lesions. *Gastrointest Endosc* 1994;40(2 Pt 1):199-206.
18. Tsang TK, Hidvegi D, Horth K, Ostrow JD: Reliability of balloon-mesh cytology in detecting esophageal carcinoma in a population of US veterans. *Cancer* 1987;59(3):556-559.
19. Yang H, Berner A, Mei Q, et al.: Cytologic screening for esophageal cancer in a high-risk population in Anyang County, China. *Acta Cytol* 2002;46(3):445-452.
20. Sepehr A, Razavi P, Saidi F, et al.: Esophageal exfoliative cytology samplers. A comparison of three types. *Acta Cytol* 2000;44(5):797-804.
21. Wang L, Yang H, Fan Z, et al.: Cytological screening and 15 years' follow-up (1986-2001) for early esophageal squamous cell carcinoma and precancerous lesions in a high-risk population in Anyang County, Henan Province, Northern China. *Cancer Detection and Prevention* 2005;29(4):317-322.
22. Cusso X, Mones J, Ocana J, et al.: Is endoscopic gastric cytology worthwhile? An evaluation of 903 cases of carcinoma. *J Clin Gastroenterol* 1993;16(4):336-339.
23. Wang HH, Sovie S, Trawinski G, et al.: ThinPrep processing of endoscopic brushing specimens. *Am J Clin Pathol* 1996;105(2):163-167.
24. Shanghai Gastrointestinal Endoscopy Cooperative Group PsRoC: Value of biopsy and brush cytology in the diagnosis of gastric cancer. *Gut* 1982;23(9):774-776.
25. Behmard S, Sadeghi A, Bagheri SA: Diagnostic accuracy of endoscopy with brushing cytology and biopsy in upper gastrointestinal lesions. *Acta Cytol* 1978;22(3):153-154.
26. Cook IJ, de Carle DJ, Haneman B, et al.: The role of brushing cytology in the diagnosis of gastric malignancy. *Acta Cytol* 1988;32(4):461-464.
27. Geisinger KR: Endoscopic biopsies and cytologic brushings of the esophagus are diagnostically complementary. *Am J Clin Pathol* 1995;103:295-299.
28. Kobayashi S, Kasugai T: Brushing cytology for the diagnosis of gastric cancer involving the cardia or the lower esophagus. *Acta Cytol* 1978;22(3):155-157.
29. Lan CS: Critical evaluation of the cytodiagnosis of fibrogastroendoscopic samples obtained under direct vision. *Acta Cytol* 1990;34(2):217-220.
30. O'Donoghue JM, Horgan PG, O'Donohue MK, et al.: Adjunctive endoscopic brush cytology in the detection of upper gastrointestinal malignancy. *Acta Cytol* 1995;39(1):28-34.
31. Prolla JC, Yoshii Y, Kirsner JB, Xavier RG: Further experience with direct vision brushing cytology of malignant tumors of upper gastrointestinal tract histopathologic correlation with biopsy. *Acta Cytol* 1971;15(4):375-378.
32. Qizilbash AH, Castelli M, Kowalski MA, Churly A: Endoscopic brush cytology and biopsy in the diagnosis of cancer of the upper gastrointestinal tract. *Acta Cytol* 1980;24(4):313-318.
33. Witzel L, Halter F, Gretillat PA, et al.: Evaluation of specific value of endoscopic biopsies and brush cytology for malignancies of the oesophagus and stomach. *Gut* 1976;17(5):375-377.
34. Young JA, Hughes HE: Three year trial of endoscopic cytology of the stomach and duodenum. *Gut* 1980;21(3):241-246.
35. Geramizadeh B, Shafiee A, Saberfirruzi M, et al.: Brush cytology of gastric malignancies. *Acta Cytol* 2002;46(4):693-696.
36. Keighley M, Thompson H, Moore J, et al.: Comparison of brush cytology before or after biopsy for diagnosis of gastric carcinoma. *Br J Surg* 1979;66(4):246-247.
37. Zargar SA, Khuroo MS, Jan GM, et al.: Prospective comparison of the value of brushings before and after biopsy in the endoscopic diagnosis of gastroesophageal malignancy. *Acta Cytol* 1991;35(5):549-552.
38. Wang HH, Doria MI, Purohit-Buch S, et al.: Barrett's esophagus. The cytology of dysplasia in comparison to benign and malignant lesions. *Acta Cytol* 1992;36(1):60-64.
39. Geisinger K: Cytology in Barrett's esophagus. *Diagn Cytopathol* 1998;19:401-402.
40. Falk G: Cytology in Barrett's esophagus. *Gastroenterol Clin North Am* 2003;13(2):335-348.
41. Saad RS, Mahood LK, Clary KM, et al.: Role of cytology in the diagnosis of Barrett's esophagus and associated neoplasia. *Diagn Cytopathol* 2003;29(3):130-135.
42. Dawsey SM, Yu Y, Taylor PR, et al.: Esophageal cytology and subsequent risk of esophageal cancer. A prospective follow-up study from Linxian, China. *Acta Cytol* 1994;38(2):183-192.
43. Dawsey SM, Shen Q, Nieberg RK, et al.: Studies of esophageal balloon cytology in Linxian, China. *Cancer Epidemiol Biomarkers Prev* 1997;6(2):121-130.
44. Liu SE, Shen Q, Dawsey SM, et al.: Esophageal balloon cytology and subsequent risk of esophageal and gastric-cardia cancer in a high-risk Chinese population. *Int J Cancer* 1994;57(6):775-780.
45. Alexander JA, Jones SM, Smith CJ, et al.: Usefulness of cytopathology and histology in the evaluation of Barrett's esophagus in a community hospital. *Gastrointest Endosc* 1997;46(4):318-320.
46. Baehr P, McDonald G: Esophageal infections: Risk factors, presentations, diagnosis, and treatment. *Gastroenterology* 1994;106:509-532.

47. Wilcox C, Karowe M: Esophageal infections: Etiology, diagnosis, and management. *Gastroenterologist* 1994;2:188-206.
48. O'Morchoe PJ, Lee DC, Kozak CA: Esophageal cytology in patients receiving cytotoxic drug therapy. *Acta Cytol* 1983;27(6):630-634.
49. Thom K, Forrest G: Gastrointestinal infections in immunocompromised hosts. *Curr Opin Gastroenterol* 2006;22(1):18-23.
50. Feiden W, Borchard F, Burring K, Pfitzer P: Herpes oesophagitis. I. Light microscopical and immunohistochemical investigations. *Virchows Arch* 1984;404(2):167-176.
51. Wilcox CM, Diehl D, Cello J, et al.: Cytomegalovirus esophagitis in patients with AIDS. A clinical, endoscopic, and pathologic correlation. *Ann Intern Med* 1990;113(8):589-593.
52. Muir SW, Murray J, Farquharson MA, et al.: Detection of cytomegalovirus in upper gastrointestinal biopsies from heart transplant recipients: Comparison of light microscopy, immunocytochemistry, in situ hybridisation, and nested PCR. *J Clin Pathol* 1998;51(11):807-811.
53. Norton RA, Caserta MT, Hall CB, et al.: Detection of human herpesvirus 6 by reverse transcription-PCR. *J Clin Microbiol* 1999;37(11):3672-3675.
54. Persons DL, Moore JA, Fishback JL: Comparison of polymerase chain reaction, DNA hybridization, and histology with viral culture to detect cytomegalovirus in immunosuppressed patients. *Mod Pathol* 1991;4(2):149-153.
55. Spechler SJ: Barrett's esophagus. *Semin Gastrointest Dis* 1996;7(2):51-60.
56. Spechler SJ: Barrett's Esophagus. *N Engl J Med* 2002;346(11):836-842.
57. Sarr MG, Hamilton SR, Marrone GC, Cameron JL: Barrett's esophagus: Its prevalence and association with adenocarcinoma in patients with symptoms of gastroesophageal reflux. *Am J Surg* 1985;149(1):187-193.
58. Cameron AJ, Zinsmeister AR, Ballard DJ, Carney JA: Prevalence of columnar-lined (Barrett's) esophagus. Comparison of population-based clinical and autopsy findings [see comments]. *Gastroenterology* 1990;99(4):918-922.
59. Thompson JJ, Zinsser KR, Enterline HT: Barrett's metaplasia and adenocarcinoma of the esophagus and gastroesophageal junction. *Hum Pathol* 1983;14(1):42-61.
60. Enzinger PC, Mayer RJ: Esophageal cancer. *N Engl J Med* 2003;349(23):2241-2252.
61. Shaheen N, Ransohoff DF: Gastroesophageal reflux, Barrett esophagus, and esophageal cancer. Scientific review. *JAMA* 2002;287:1972-1986.
62. Spechler SJ: Endoscopic surveillance for patients with Barrett esophagus: Does the cancer risk justify the practice? *Ann Intern Med* 1987;106(6):902-904.
63. Haggitt RC: Barrett's esophagus, dysplasia, and adenocarcinoma. *Hum Pathol* 1994;25(10):982-993.
64. Ruol A, Parenti A, Zaninotto G, et al.: Intestinal metaplasia is the probable common precursor of adenocarcinoma in Barrett esophagus and adenocarcinoma of the gastric cardia. *Cancer* 2000;88(11):2520-2528.
65. Sharma P, Morales TG, Sampliner RE: Short segment Barrett's esophagus—the need for standardization of the definition and of endoscopic criteria. *Am J Gastroenterol* 1998;93(7):1033-6.
66. Genta RM, Huberman RM, Graham DY: The gastric cardia in *Helicobacter pylori* infection. *Hum Pathol* 1994;25(25):915-919.
67. MacLennan AJ, Orringer MB, Beer DG: Identification of intestinal-type Barrett's metaplasia by using the intestine-specific protein villin and esophageal brush cytology. *Mol Carcinog* 1999;24(2):137-143.
68. Zhang J, Parwani AV, Ali SZ: Hepatocyte paraffin 1 immunorexpression in esophageal brush samples. *Cancer* 2005;105(5):304-309.
69. Smith RR, Hamilton SR, Boitnott JK, Rogers EL: The spectrum of carcinoma arising in Barrett's esophagus. A clinicopathologic study of 26 patients. *Am J Surg Pathol* 1984;8(8):563-573.
70. Montgomery E, Bronner MP, Goldblum JR, et al.: Reproducibility of the diagnosis of dysplasia in Barrett esophagus: A reaffirmation. *Hum Pathol* 2001;32(4):368-378.
71. Wang HH, Ducatman BS, Thibault S: Cytologic features of premalignant glandular lesions in the upper gastrointestinal tract. *Acta Cytol* 1991;35(2):199-203.
72. Rusch VW, Levine DS, Haggitt R, Reid BJ: The management of high grade dysplasia and early cancer in Barrett's esophagus. A multidisciplinary problem. *Cancer* 1994;74(4):1225-1229.
73. Heitmiller RE, Redmond M, Hamilton SR: Barrett's esophagus with high-grade dysplasia. An indication for prophylactic esophagectomy. *Ann Surg* 1996;224(1):66-71.
74. Hughes JH, Cohen MB: Is the cytologic diagnosis of esophageal glandular dysplasia feasible? [see comments]. *Diagn Cytopathol* 1998;18(4):312-316.
75. Jankowski JA, Wright NA, Meltzer SJ, et al.: Molecular evolution of the metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. *Am J Pathol* 1999;154(4):965-973.
76. Nobukawa B, Abraham SC, Gill J, et al.: Clinicopathologic and molecular analysis of high-grade dysplasia and early adenocarcinoma in short- versus long-segment Barrett esophagus. *Hum Pathol* 2001;32(4):447-454.
77. Bian YS, Osterheld MC, Bosman FT, et al.: p53 gene mutation and protein accumulation during neoplastic progression in Barrett's esophagus. *Mod Pathol* 2001;14(5):397-403.
78. Gimenez A, de Haro LM, Parrilla P, et al.: Immunohistochemical detection of p53 protein could improve the management of some patients with Barrett esophagus and mild histologic alterations. *Arch Pathol Lab Med* 1999;123(12):1260-1263.
79. Hanas JS, Lerner MR, Lightfoot SA, et al.: Expression of the cyclin-dependent kinase inhibitor p21(WAF1/CIP1) and p53 tumor suppressor in dysplastic progression and adenocarcinoma in Barrett esophagus. *Cancer* 1999;86(5):756-763.
80. Jones DR, Davidson AG, Summers CL, et al.: Potential application of p53 as an intermediate biomarker in Barrett's esophagus. *Ann Thorac Surg* 1994;57(3):598-603.
81. Devesa SS, Blot WJ, Fraumeni JF Jr: Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer* 1998;83(10):2049-2053.
82. Shaheen N: Is there a "Barrett's iceberg?" *Gastroenterology* 2002;123(2):636-639.
83. Thomas RM, Sobin LH: Gastrointestinal cancer. *Cancer* 1995;75(1 Suppl):154-170.
84. Dooley CP, Cohen H, Fitzgibbons PL, et al.: Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. *N Engl J Med* 1989;321(23):1562-1566.
85. Parsonnet J, Friedman GD, Vandersteen DP, et al.: *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 1991;325(16):1127-1131.
86. Parsonnet J, Hansen S, Rodriguez L, et al.: *Helicobacter pylori* infection and gastric lymphoma. *N Engl J Med* 1994;330(18):1267-1271.
87. Isaacson PG, Du MQ: MALT lymphoma: from morphology to molecules. *Nat Rev Cancer* 2004;4(8):644-653.
88. Senturk O, Canturk Z, Ercin C, et al.: Comparison of five detection methods for *Helicobacter pylori*. *Acta Cytol* 2000;44(6):1010-1014.
89. Schnadig VJ, Bigio EH, Gourley WK, et al.: Identification of *Campylobacter pylori* by endoscopic brush cytology. *Diagn Cytopathol* 1990;6(4):227-234.
90. Faraker C: Diagnosis of *Helicobacter pylori* in gastric brush and biopsy specimens stained by Romanowsky and immunocytochemical methods: comparison with the CLOtest. *Cytopathology* 1996;7:108-119.

91. Mendoza ML, Martin-Rabadian P, Carrion I, et al.: *Helicobacter pylori* infection. Rapid diagnosis with brush cytology. *Acta Cytol* 1993;37(2):181-185.
92. Cubukcu A, Gonullu NN, Ercin C, et al.: Imprint cytology in the diagnosis of *Helicobacter pylori*. Does imprinting damage the biopsy specimen? *Acta Cytol* 2000;44(2):124-127.
93. Narvaez Rodriguez I, Saez de Santamaria J, Alcalde Rubio MM, et al.: Cytologic brushing as a simple and rapid method in the diagnosis of *Helicobacter pylori* infection. *Acta Cytol* 1995;39(5):916-919.
94. Ghossoub RA, Lachman MF: A triple stain for the detection of *Helicobacter pylori* in gastric brushing cytology. *Acta Cytol* 1997;41(4):1178-1182.
95. Maygarden SJ, Flanders EL: Mycobacteria can be seen as "negative images" in cytology smears from patients with acquired immunodeficiency syndrome. *Mod Pathol* 1989;2(3):239-243.
96. Jensen RT: Carcinoid and pancreatic endocrine tumors: recent advances in molecular pathogenesis, localization, and treatment. *Curr Opin Oncol* 2000;12(4):368-377.
97. Caplin M, Buscombe J, Hilson A, et al.: Carcinoid tumour. *Lancet* 1998;352(9130):799-805.
98. DeLellis RA: The neuroendocrine system and its tumors. An overview. *Am J Clin Pathol* 2001;115(Suppl 1):S5-S16.
99. Fenoglio-Preiser CM: Gastrointestinal neuroendocrine/neuroectodermal tumors. *Am J Clin Pathol* 2001;115(Suppl 1):S79-S93.
100. Modlin IM, Lye KD, Kidd M: A 5-decade analysis of 13,715 carcinoid tumors. *Cancer* 2003;97(4):934-959.
101. Modlin IM, Sandor A: An analysis of 8305 cases of carcinoid tumors. *Cancer* 1997;79(4):813-829.
102. Sevrerson RK, Davis S: Increasing incidence of primary gastric lymphoma. *Cancer* 1990;66(6):1283-1287.
103. Greiner TC, Medeiros LJ, Jaffe ES: Non-Hodgkin's lymphoma. *Cancer* 1995;75(1 Suppl):370-380.
104. Morgner A, Bayerdorffer E, Neubauer A, Stolte M: Malignant tumors of the stomach. Gastric mucosa-associated lymphoid tissue lymphoma and *Helicobacter pylori*. *Gastroenterol Clin North Am* 2000;29(3):593-607.
105. Chan JK, Ng CS, Isaacson PG: Relationship between high-grade lymphoma and low-grade B-cell mucosa-associated lymphoid tissue lymphoma (MALToma) of the stomach. *Am J Pathol* 1990;136(5):1153-1164.
106. Chuang SS, Lee C, Hamoudi RA, et al.: High frequency of t(11;18) in gastric mucosa-associated lymphoid tissue lymphomas in Taiwan, including one patient with high-grade transformation. *Br J Haematol* 2003;120(1):97-100.
107. Du MQ, Diss TC, Dogan A, et al.: Clone-specific PCR reveals wide dissemination of gastric MALT lymphoma to the gastric mucosa. *J Pathol* 2000;192(4):488-493.
108. Harris NL, Jaffe ES, Diebold J, et al.: The World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. Report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November, 1997. *Ann Oncol* 1999;10(12):1419-1432.
109. d'Amore F, Brincker H, Gronbaek K, et al.: Non-Hodgkin's lymphoma of the gastrointestinal tract: a population-based analysis of incidence, geographic distribution, clinicopathologic presentation features, and prognosis. Danish Lymphoma Study Group. *J Clin Oncol* 1994;12(8):1673-1684.
110. Koch P, del Valle F, Berdel WE, et al.: Primary gastrointestinal non-Hodgkin's lymphoma: I. Anatomic and histologic distribution, clinical features, and survival data of 371 patients registered in the German Multicenter Study GIT NHL 01/92. *J Clin Oncol* 2001;19(18):3861-3873.
111. Nakamura S, Akazawa K, Yao T, Tsuneyoshi M: A clinicopathologic study of 233 cases with special reference to evaluation with the MIB-1 index. *Cancer* 1995;76(8):1313-1324.
112. Chhieng DC, Cohen JM, Cangiarella JF: Cytology and immunophenotyping of low- and intermediate-grade B-cell non-Hodgkin's lymphomas with a predominant small-cell component: a study of 56 cases. *Diagn Cytopathol* 2001;24(2):90-97.
113. Crapanzano JP, Lin O: Cytologic findings of marginal zone lymphoma. *Cancer* 2003;99(5):301-309.
114. Vander Noot MR 3rd, Eloubeidi MA, et al.: Diagnosis of gastrointestinal tract lesions by endoscopic ultrasound-guided fine-needle aspiration biopsy. *Cancer* 2004;102(3):157-163.
115. Arantes V, Logrono R, Faruqi S, et al.: Endoscopic sonographically guided fine-needle aspiration yield in submucosal tumors of the gastrointestinal tract. *J Ultrasound Med* 2004;23(9):1141-1150.
116. Lozowski W, Hajdu SI: Preoperative cytologic diagnosis of primary gastrointestinal malignant lymphoma. *Acta Cytol* 1984;28(5):563-570.
117. Miettinen M, Sarlomo-Rikala M, Lasota J: Gastrointestinal stromal tumors: recent advances in understanding of their biology. *Hum Pathol* 1999;30(10):1213-1220.
118. Sircar K, Hewlett BR, Huizinga JD, et al.: Interstitial cells of Cajal as precursors of gastrointestinal stromal tumors. *Am J Surg Pathol* 1999;23(4):377-389.
119. Fletcher CD, Berman JJ, Corless C, et al.: Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Int J Surg Pathol* 2002;10(2):81-89.
120. Stelow EB, Stanley MW, Mallory S, et al.: Endoscopic ultrasound-guided fine-needle aspiration findings of gastrointestinal leiomyomas and gastrointestinal stromal tumors. *Am J Clin Pathol* 2003;119(5):703-708.
121. Dodd LG, Nelson RC, Mooney EE, Gottfried M: Fine-needle aspiration of gastrointestinal stromal tumors. *Am J Clin Pathol* 1998;109(4):439-443.
122. Wieczorek TJ, Faquin WC, Rubin BP, Cibas ES: Cytologic diagnosis of gastrointestinal stromal tumor with emphasis on the differential diagnosis with leiomyosarcoma. *Cancer* 2001;93(4):276-287.
123. Rader AE, Avery A, Wait CL, et al.: Fine-needle aspiration biopsy diagnosis of gastrointestinal stromal tumors using morphology, immunocytochemistry, and mutational analysis of c-kit. *Cancer* 2001;93(4):269-275.
124. Boggino HE, Fernandez MP, Logrono R: Cytomorphology of gastrointestinal stromal tumor: Diagnostic role of aspiration cytology, core biopsy, and immunochemistry. *Diagn Cytopathol* 2000;23(3):156-160.
125. Li SQ, O'Leary TJ, Sobin LH, et al.: Analysis of KIT mutation and protein expression in fine needle aspirates of gastrointestinal stromal/smooth muscle tumors. *Acta Cytol* 2000;44(6):981-986.
126. Marshall JB, Kelley DH, Vogeles KA: Giardiasis: Diagnosis by endoscopic brush cytology of the duodenum. *Am J Gastroenterol* 1984;79(7):517-519.
127. Bendig D: Diagnosis of giardiasis in infants and children by endoscopic brush cytology. *J Pediatr Gastroenterol Nutr* 1989;8(2):204-206.
128. Chu P, West AB: Encephalitozoon (Septata) intestinalis: cytologic, histologic, and electron microscopic features of a systemic intestinal pathogen. *Am J Clin Pathol* 1996;106(5):606-614.
129. Pol S, Romana CA, Richard S, et al.: Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med* 1993;328(2):95-99.
130. Weber R, Bryan RT, Owen RL, et al.: Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. The Enteric Opportunistic Infections Working Group. *N Engl J Med* 1992;326(3):161-166.

131. Clayton F, Heller T, Kotler DP: Variation in the enteric distribution of *Cryptosporidia* in acquired immunodeficiency syndrome. *Am J Clin Pathol* 1994;102(4):420-425.
132. Yamaguchi K, Enjoji M: Carcinoma of the ampulla of Vater. A clinicopathologic study and pathologic staging of 109 cases of carcinoma and 5 cases of adenoma. *Cancer* 1987;59(3):506-515.
133. Ryan DP, Schapiro RH, Warshaw AL: Villous tumors of the duodenum. *Ann Surg* 1986;203(3):301-306.
134. Agoff S, Crispin D, Bronner M, et al.: Neoplasms of the ampulla of Vater with concurrent pancreatic intraductal neoplasia: a histological and molecular study. *Mod Pathol* 2001;14(3):139-146.
135. Bardales RH, Stanley MW, Simpson DD, et al.: Diagnostic value of brush cytology in the diagnosis of duodenal, biliary, and ampullary neoplasms. *Am J Clin Pathol* 1998;109(5):540-548.
136. Isbister WH, Gupta RK: Colonoscopy, mucosal biopsy and brush cytology in the assessment of patients with colorectal inflammatory bowel disease. *Surg Endosc* 1989;3(3):159-163.
137. Melville DM, Richman PI, Shepherd NA, et al.: Brush cytology of the colon and rectum in ulcerative colitis: An aid to cancer diagnosis. *J Clin Pathol* 1988;41(11):1180-1186.
138. Boddington MM, Truelove SC: Abnormal epithelial cells in ulcerative colitis. *Br Med J* 1956;1:1318-1321.
139. Festa VI, Hajdu SI, Winawer SJ: Colorectal cytology in chronic ulcerative colitis. *Acta Cytol* 1985;29(3):262-268.
140. Galambos JT, Massey BW, Klayman MI, Kirsner JB: Exfoliative cytology in chronic ulcerative colitis. *Cancer* 1956;9:152-159.
141. Bardawil RG, D'Ambrosio FG, Hajdu SI: Colonic cytology. A retrospective study with histopathologic correlation. *Acta Cytol* 1990;34(5):620-626.
142. Ehya H, O'Hara BJ: Brush cytology in the diagnosis of colonic neoplasms. *Cancer* 1990;66(7):1563-1567.
143. Halpern M, Gal R, Rath-Wolfson L, et al.: Brush cytology and biopsy in the diagnosis of colorectal cancer. A comparison. *Acta Cytol* 1997;41(3):628-632.
144. Marshall JB, Diaz-Arias AA, Barthel JS, et al.: Prospective evaluation of optimal number of biopsy specimens and brush cytology in the diagnosis of cancer of the colorectum. *Am J Gastroenterol* 1993;88(9):1352-1354.
145. Petrelli NJ, Letourneau R, Weber T, et al.: Accuracy of biopsy and cytology for the preoperative diagnosis of colorectal adenocarcinoma. *J Surg Oncol* 1999;71(1):46-49.
146. Yu GH, Nayar R, Furth EE: Adenocarcinoma in colonic brushing cytology: High-grade dysplasia as a diagnostic pitfall. *Diagn Cytopathol* 2001;24(5):364-368.
147. Daling JR, Weiss NS, Klopfenstein LL, et al.: Correlates of homosexual behavior and the incidence of anal cancer. *JAMA* 1982;247(14):1988-1990.
148. Chiao EY, Giordano TP, Palefsky JM, et al.: Screening HIV-infected individuals for anal cancer precursor lesions: A systematic review. *Clin Infect Dis* 2006;43(2):223-233.
149. Jemal A, Siegel R, Ward E, et al.: Cancer statistics, 2007. *CA Cancer J Clin* 2007;57(1):43-66.
150. Solomon D, Nayar R: *The Bethesda System for Reporting Cervical Cytology*, 2nd ed. New York, Springer, 2004.

Breast

BARBARA S. DUCATMAN and HELEN H. WANG

SPECIMEN TYPES

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UNCOMMON BREAST TUMORS

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METASTATIC TUMORS

The most common cytologic specimen of the breast is the fine-needle aspiration (FNA). FNA is a widely used method for evaluating palpable and radiologically directed nonpalpable breast abnormalities. FNA is still employed despite competition from the automated core needle biopsy (CNB) under stereotactic mammographic guidance, a highly accurate technique that is preferred by many pathologists.¹⁻³ For nonpalpable lesions, FNA is a useful technique for sampling cystic lesions under ultrasound guidance, whereas CNB is more often used for calcifications noted in mammograms.⁴

The main advantage of FNA is immediate sample assessment for adequacy.⁵ Although a touch imprint of a CNB can be done for rapid diagnosis, the usefulness of this approach is controversial.⁶⁻¹² Compared with CNB, the nondiagnostic rate for FNA is higher, and FNA has a lower negative predictive value.^{5,12,13} Nevertheless, some authors have reported excellent results with breast FNA and the use of a combination of FNA and CNB may be superior to either FNA or CNB alone.¹⁴⁻²⁰ CNB and FNA are each less expensive than excisional biopsy.^{21,22}

Although atypical ductal proliferative lesions are more amenable to classification by CNB than by FNA, false-negative diagnoses or underestimates of malignancy have been reported in a considerable percentage of cases

even with CNB.²³⁻²⁶ Prognostic markers can be assessed using either technique.²⁷ Many practitioners, however, have recommended that CNB replace FNA^{13,28-30} because of the increased negative predictive value and wider acceptance of histopathology as compared to cytopathology. Indeed, the increasing use of CNB, particularly with stereotactic or ultrasound guidance, has led to diminished use of FNA,^{20,31} although some continue to use FNA for patients with radiologically and clinically negative lesions.^{2,32}

SPECIMEN TYPES

Nipple Discharge, Nipple Aspiration, and Ductal Lavage

A spontaneous nipple discharge not related to lactation or pregnancy is an abnormal finding. It may result from a lesion in the breast, such as a papilloma or a carcinoma, or from a hormonal abnormality, such as a prolactin-secreting pituitary adenoma. Cytologic examination of a nipple discharge is generally used when the patient has no palpable or mammographic abnormality. It is useful in identifying minimal breast cancers and papillomatosis.^{33,34} If a palpable or mammographic abnormality is present, a needle (FNA or CNB) or excisional biopsy is usually performed.

The sensitivity of nipple discharge cytology ranges from 41% to 60%.³⁵⁻³⁷ Nipple discharge cytology is not useful as a screening test for breast cancer because a discharge can be obtained in only 7% to 14% of women who are asymptomatic, not pregnant, and not lactating.^{35,37,38} Biomarker analysis of nipple discharge fluid may be used instead of or in addition to cytology in the future to increase sensitivity.³⁹

Nipple discharge specimens are prepared by gently massaging the breast in the direction of the nipple. A glass slide is then touched to the secreted drops; the discharge need not be smeared unless it is abundant. The slides are fixed by spray fixation or by immersion in 95% ethyl alcohol and then stained with the Papanicolaou stain. An alternative method is to air-dry the slide and stain it with a Romanowsky-type stain.

A nipple discharge can be unilateral or bilateral; unilateral discharges are more likely to be malignant.³⁵ The secretion may be milky, serous, purulent, or bloody. Cancer is most prevalent when the discharge is macroscopically bloody (4%) and less prevalent when it is purulent (0.8%), serous (0.2%), or milky (0.1%).³⁵ Because bloody discharges are more likely than nonbloody discharges to contain malignant cells, some authors recommend cytologic examination of bloody secretions only, which represent 11% of all secretions.³⁵ Others recommend examination of all discharges.³⁷ Most patients with an intraductal papilloma do have a discharge,³⁷ which may or may not be bloody. Although most patients with breast cancer do not have a discharge from the nipple, about 2% of breast cancers are detected by this method and by no other.³⁷

In the absence of a spontaneous discharge, fluid can be aspirated from the nipple by applying suction with a breast pump. Nipple aspirate fluid (NAF) can be evaluated for cytomorphology and for a host of biomarkers.^{39,40} A long-term followup study of 3663 women from whom NAF was collected with a breast pump demonstrated an increased risk of breast cancer in those women with abnormal cytology.⁴¹

Atypical and malignant NAF cytology results in women with biopsy-proven invasive or in situ ductal carcinoma are associated with larger tumor size and are predictive of residual carcinoma after needle or excisional biopsy of the breast.⁴² Newer methods of harvesting a NAF to screen asymptomatic women, such as the intraductal catheter, compare favorably to traditional nipple squeezing, with significantly improved adequacy (96.6% versus 76%), sensitivity (92.3% versus 52.9%) and specificity (93.9% versus 89.3%).⁴³

CYTOMORPHOLOGY OF BENIGN NIPPLE SECRETIONS AND ASPIRATIONS:

- usually sparsely cellular
- ductal cells
- foam cells
- inflammatory cells
- red blood cells

Benign ductal cells are arranged in tight clusters that are small and spherical or large and branching; isolated ductal cells are uncommon. Usually, the cells are small and have scant cytoplasm, but occasionally they are larger and have abundant cytoplasm. It is common for benign ductal cells to mold themselves around one another, giving the clusters a scalloped appearance. Foam cells are large histiocytes that are usually dispersed as isolated cells. They contain abundant vacuolated cytoplasm and a round or oval nucleus that is sometimes degenerated (Fig. 8.1A). When a secretion contains numerous groups of benign ductal cells, especially large, branching clusters, it is likely that the patient has an intraductal papilloma or a florid intraductal hyperplasia, lesions that can only be distinguished histologically.

In the future, NAF cytology will likely compete with an array of new tests (e.g., microbiopsy), and widespread

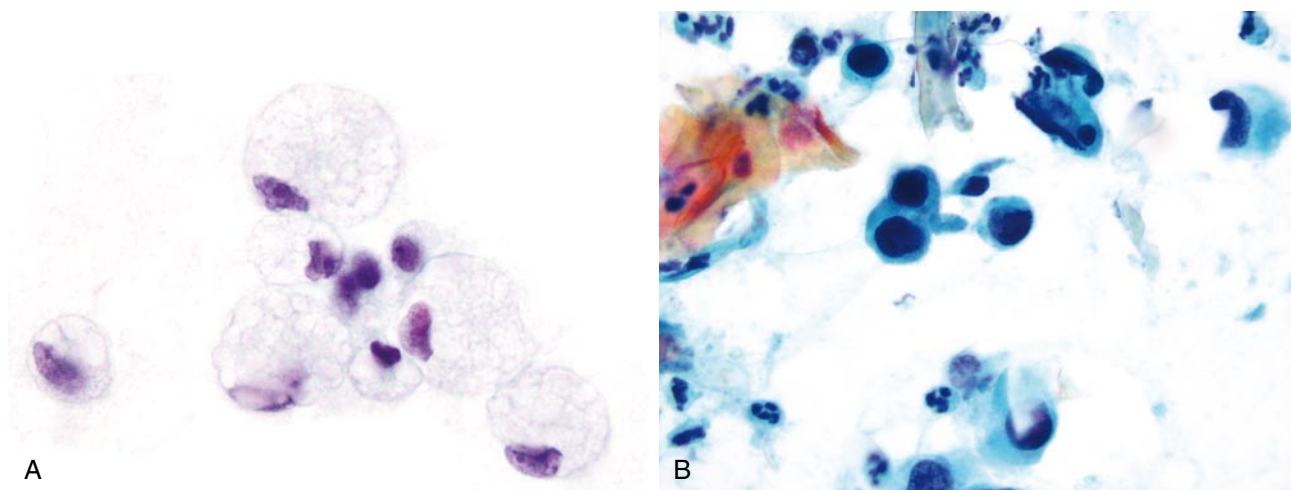


Figure 8.1 Nipple discharge cytology. A, Benign nipple discharge. Histiocytes (foam cells) have a kidney-bean-shaped nucleus and abundant vacuolated cytoplasm (Papanicolaou stain). B, Suspicious nipple discharge. The sample contains atypical cells with enlarged nuclei. The subsequent biopsy showed a high-grade comedocarcinoma.

CYTOMORPHOLOGY OF MALIGNANT NIPPLE SECRETIONS AND ASPIRATIONS:

- clusters and single enlarged ductal cells
- variable nuclear size and shape
- stripped nuclei
- nucleoli
- acute inflammation
- blood
- necrotic debris
- [Figure 8.1B](#)

adoption of it for breast cancer risk assessment is still controversial.^{44,45}

Ductal lavage is a newer, more invasive method of sampling the ductal epithelium.⁴⁶⁻⁴⁸ It involves infusing 10 to 20 mL of saline into the duct through an inserted microcatheter. The saline “rinse,” combined with breast compression, enables collection of breast ductal epithelial cells. The collection is deposited into preservative and commonly prepared as a ThinPrep slide.⁴⁹

The interpretation of ductal lavage fluid specimens is based on the morphology of ductal cells.⁵⁰ The diagnosis is classified into one of the following categories: insufficient cellular material for diagnosis (fewer than 10 epithelial cells), benign epithelial cells, mild ductal cell atypia, marked ductal cell atypia, and malignant.

CYTOMORPHOLOGY OF BENIGN DUCTAL LAVAGE:

- isolated unremarkable columnar epithelial cells with moderately abundant cytoplasm
- flat ductal cell sheets
- small nucleolus, mild variation in epithelial nuclear size
- [Figure 8.2A](#)

CYTOMORPHOLOGY OF MILDLY ATYPICAL DUCTAL LAVAGE:

- three-dimensional, micropapillary clusters
- moderate nuclear enlargement
- mild anisonucleosis
- more prevalent small nucleoli
- increased nuclear-to-cytoplasmic ratio in occasional cells
- regular nuclear membrane and finely granular chromatin
- [Figure 8.2B](#)

CYTOMORPHOLOGY OF MARKEDLY ATYPICAL DUCTAL LAVAGE:

- moderate to marked cellular and nuclear enlargement
- irregular nuclear membrane
- coarsely granular chromatin
- large and variable nucleoli
- more consistent increased nuclear-to-cytoplasmic ratio
- occasional multinucleation and mitoses
- microcalcifications and necrotic debris may be noted

In benign ductal lavages, the ductal cells are isolated or arranged in monolayers. Three-dimensional clusters are considered atypical, and the distinction between mild and marked atypical changes rests on the degree of nuclear atypia. Irregular arrangements, anisonucleosis, hyperchromasia, and nuclear overlap merit the designation “marked ductal cell atypia.” Malignant cells have a similar morphology to those observed in FNA specimens.

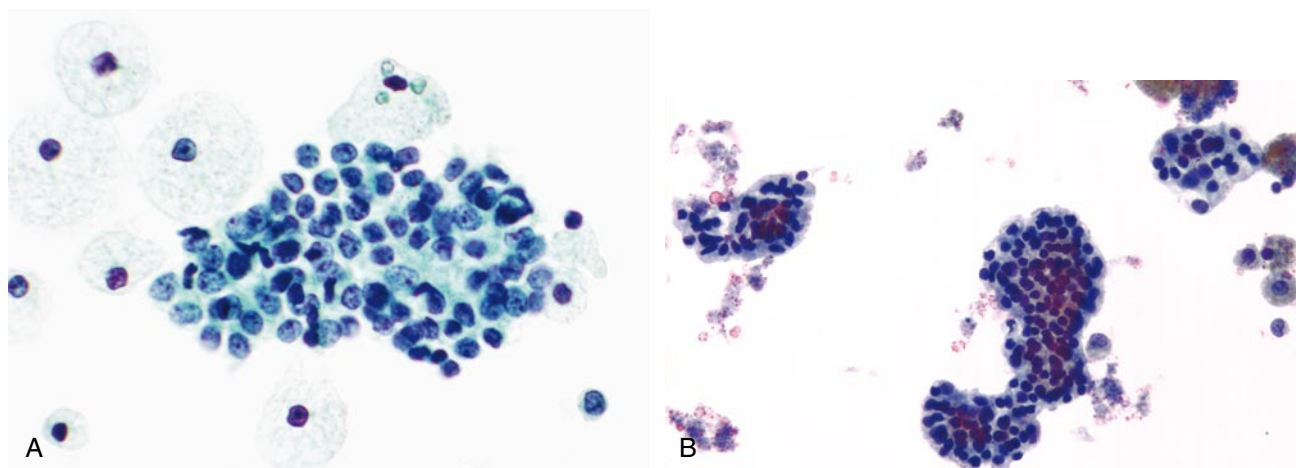


Figure 8.2 Ductal lavage specimens. A, Benign epithelial cells. Benign ductal cells with interspersed myoepithelial cells are often seen in flat sheets. B, Mild ductal cell atypia. Note the three-dimensional clusters of cells and the slight nuclear enlargement. Although this case was diagnosed as “mildly atypical,” a breast biopsy showed micropapillary ductal carcinoma in situ (DCIS), which demonstrates how challenging the diagnosis of low-grade breast lesions can be. (ThinPrep, Papanicolaou stain).

Despite initial enthusiasm for its use in risk stratification and biomarker identification,⁵¹⁻⁵³ recent studies cast doubt on the value of ductal lavage, even in the research setting.⁵⁴ Diagnosis of breast carcinoma from ductal lavage is specific but not sensitive.⁵⁵ Random periareolar FNA is superior to ductal lavage in its lower cost and higher likelihood to produce an evaluable specimen, even though both methods yield similar morphology.^{56,57} In addition, ductal lavage suffers from technical challenges in duct cannulation, inconsistent NAF production, fair cytologic reproducibility, and low participant return rates.⁵⁸

Another method is FNA via ultrasound guidance through a galactographic catheter, which is more accurate (50%) than nipple discharge cytology (25%).⁵⁹

Fine-Needle Aspiration Cytology

FNA is widely used for evaluating palpable breast masses, breast cysts, and even nonpalpable mammographic abnormalities. The use of FNA or core biopsies significantly decreases health care costs by decreasing the number of open surgical biopsies per breast cancer identified, without sacrificing early detection.²¹ When the diagnosis is benign, such as a lactating adenoma in a patient who is pregnant, FNA spares a patient with a solid and palpable lesion an open biopsy. A diagnosis of malignancy allows preoperative discussion of available therapeutic options (lumpectomy with radiation versus mastectomy), or it might persuade a reluctant patient to undergo surgical biopsy. Ultrasound-guided FNA of axillary lymph nodes is advocated as a way to triage patients for appropriate management.⁶⁰⁻⁶⁵ Patients with positive aspirates proceed directly to axillary dissection or neoadjuvant chemotherapy, whereas those with negative aspirates have sentinel lymph node mapping.

The technique for performing an FNA of a palpable breast mass is the same as for any other superficial organ (Fig. 8.3). Twenty-three-gauge or 25-gauge needles with a 10mL syringe are standard. A local anesthetic is usually not used because the swelling that results can obscure the nodule. A syringe holder is often used to stabilize the syringe or needle set-up. Because many breast lesions are densely fibrous, a larger (22-gauge needle) is preferred, and in this circumstance local anesthetic may be advisable. Because many cancers have a stellate configuration, one should aspirate the center and not the periphery of a mass. After placing the needle in the center of the mass, suction is applied via the syringe. One should release suction when blood or arterial is seen in the hub in the needle; the exception to this is with fluid-filled cysts that should be drained in their entirety with reaspiration of any residual mass. The needle is withdrawn from the mass without any vacuum suction; otherwise, the cells end up in the syringe (rather than staying in the needle) and are dried and difficult to expel onto slides.

To prepare smears, the needle is removed from the syringe and the syringe is filled with air. By reattaching the needle and pushing this air with the plunger of the syringe, a small drop of aspirated material is expressed onto each slide and smears prepared. To harvest as much of the sample as possible, the needle should be washed with a preservative solution for cell block or liquid-based preparations. Although the cytologic appearance is slightly different because clusters are more three-dimensional, nuclei are smaller and darker, and less background material is seen, the accuracy of thinlayer preparations is comparable to that obtained with direct smears.^{66,67}

Complications of FNA are rare. The most common is bleeding. Occasionally, FNA causes infarction of the lesion, particularly fibroadenomas, which can make subsequent confirmation of the diagnosis more difficult.⁶⁸

Whether patients with breast cysts need cytologic analysis is controversial. Aspiration of cysts is certainly therapeutic and collapses them. The great majority of cyst fluids are benign; only about 2% prove to be carcinoma.⁶⁹ Even those complex cystic lesions noted on ultrasound are benign; in one study only 0.3% proved to be malignant.⁷⁰ Furthermore, atypical cells can be seen in a cyst fluid, resulting in overtreatment when conservative follow-up would have been adequate.^{70,71} On the other hand, a small number of carcinomas are cystic and yield fluid that looks grossly much like that of benign cysts.⁷² If the fluid is not submitted for cytologic evaluation, these carcinomas will remain undiagnosed and untreated. It has been suggested that symptomatic complicated cysts, cystic lesions with thick indistinct walls or thick septations, intracystic masses, and predominantly solid masses with cystic degeneration are more likely to be malignant and thus need further evaluation such as FNA, CNB, or excisional biopsy.⁷³

The accuracy of FNA of the breast is highly operator dependent: Sensitivity for malignancy is high, but ranges from 65% to 98%, and specificity ranges from 34% to 100% in a variety of clinical environments.^{20,74-85} False-positive results are uncommon, occurring in 0% to 2% of cases.⁸⁶ False-suspicious results are higher, ranging from 1% to 13%. In general, the sensitivity of FNA for palpable and nonpalpable malignant lesions (i.e., those sampled with mammographic or ultrasound guidance) is comparable.⁸⁷⁻¹⁰² False-negative results occur because of errors in sampling, interpretation, or both.^{83,103} Some studies show that satisfactory specimens are more likely when pathologists rather than physicians perform the aspiration.^{83,104-109} Whether physician or pathologist, however, practice makes perfect, and the one with more FNA experience obtains the more accurate result.^{110,111} The use of p63 immunostaining has recently been proposed as an adjunct to increase the accuracy of FNA by differentiating the well-differentiated carcinomas from benign lesions.¹¹²

FNA of the breast has its limitations. Although generally quite sensitive in detecting ductal carcinomas, it cannot

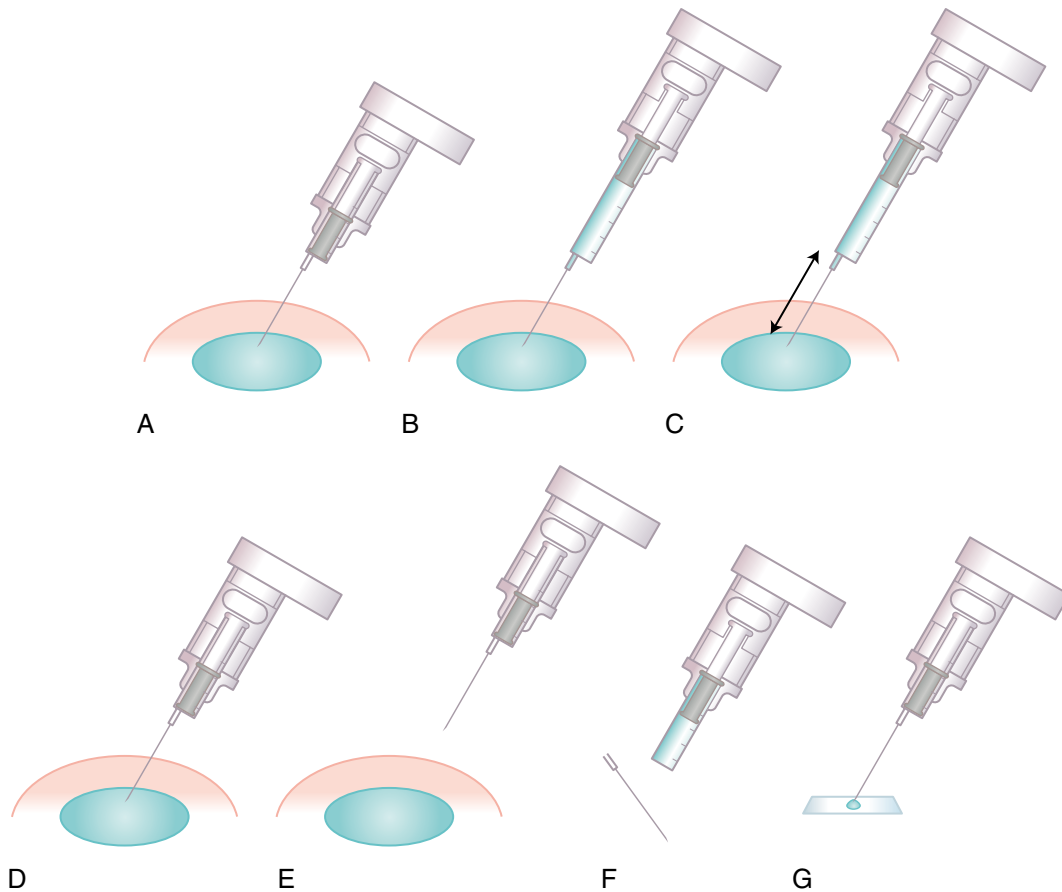


Figure 8.3 Fine-needle aspiration (FNA) procedure. The diagram demonstrates the technique for FNA of superficial palpable lesions. A, The needle is inserted into the lesion. B, Negative pressure is applied by pulling back on the plunger. C, The needle is moved rapidly back and forth in the lesion using a sawing motion. D, The plunger is released to stop the negative pressure. E, The needle is withdrawn from the patient. F, The needle is detached and air is drawn into the syringe. G, The needle is touched to a clean glass slide and the sample is expelled for smearing.

distinguish between an invasive ductal carcinoma and a ductal carcinoma in situ. It cannot identify the presence of lymphatic or vascular invasion. The diagnosis of some tumors, such as lobular carcinoma and tubular carcinoma, requires considerable experience in FNA interpretation⁹⁴; even so, equivocal findings are common because of the benign cytologic appearance of such tumors. As with FNA of other sites, considerable discrepancies exist between the performances of laboratories.¹¹³

REPORTING TERMINOLOGY

To report FNA and nipple discharge results, the use of general categories with an implicit probability or risk of malignancy, followed by a specific diagnosis, is recommended.^{2,114-121} As might be expected, interobserver reproducibility is excellent for the positive category, poor for atypical, and fair to good for other categories.¹²²

General categories for breast fine-needle aspiration:

- negative for malignant cells (no malignant cells seen)
- atypical
- suspicious
- positive for malignant cells
- non-diagnostic (inadequate or unsatisfactory)

The exact wording for each category or diagnosis is less important than having a probabilistic (risk-based) system that clearly communicates the likelihood of malignancy. One pathologist might use the term *non-diagnostic* and another, *insufficient*, but the fact that this specimen was unsatisfactory is usually obvious to physician and pathologist alike. Non-diagnostic specimens contain too few well-preserved cells to permit an adequate evaluation—fewer than six epithelial cell clusters

of at least 5 to 10 cells or less than 10 intact bipolar cells per 10 medium-power fields ($\times 200$).^{123,124}

A negative (benign) diagnosis should be reserved for an adequate specimen with a minimum of five to six well-preserved cell groupings. A negative FNA result is more reliable when a specific diagnosis corroborates a clinical and radiological impression (e.g., fibroadenoma, lactating adenoma).¹¹⁴ The physician should always correlate the cytologic result with the clinical finding and the mammographic impression (the triple test) to reduce the risk of an undiagnosed malignancy and close follow-up should ensue.¹²⁴⁻¹²⁶ The false-negative rate is greatly reduced in patients with a triple-negative test.¹²⁷ Needless to say, a negative cytologic result is by no means a guarantee that the nodule is benign, and a mammographically or clinically suspicious lesion requires a biopsy despite a negative cytologic result.

The atypical category is used for a specimen with a low probability of malignancy. This category is unavoidable because of the significant overlap in the cytologic features of some benign and malignant entities. It generally requires further biopsy assessment.¹²⁸

The suspicious diagnosis is used for lesions that are probably malignant, but the atypical cells are too few, too poorly preserved, or too obscured by blood or inflammation for a definitive diagnosis, or when the findings suggest a type of breast cancer with minimal cytologic atypia, such as lobular carcinoma, tubular carcinoma, or papillary carcinoma. Obviously, surgical biopsy should follow any specimen deemed suspicious.

Positive diagnoses are reserved for specimens with unequivocal features of malignancy. Although confirmation of all positive results with frozen section before definitive surgery is wise, some surgeons proceed directly to mastectomy or wide local excision. Therefore, it is prudent to diagnose any case for which the pathologist is not comfortable with definitive surgery as "suspicious."

EVALUATION OF THE SPECIMEN

Low-power evaluation:

- cellularity
- cellular arrangements
- background elements

Cellularity is important, although there is considerable overlap between categories. Hypocellular aspirates can be obtained from a fibroadenoma, fibrocystic changes (FCC), fat necrosis, radiation changes, pregnancy or lactation, and carcinoma, both in situ and invasive (particularly scirrhous, tubular, and lobular types). Moderately cellular aspirates are seen with a fibroadenoma, phyllodes tumor, pregnancy or lactation, FCC,

and carcinoma. Hypercellular aspirates are seen in some fibroadenomas, phyllodes tumors, and invasive carcinomas.

Cellular arrangements provide important clues to the diagnosis. Cells can be arranged in sheets, tightly or loosely cohesive three-dimensional clusters, branching papillary clusters, or as isolated cells. The spacing of nuclei in cell clusters varies in different breast lesions. Regular nuclear spacing suggests a benign process; irregular spacing is characteristic of malignancy.

Lesions associated with architectural patterns:

Sheets:

- FCC
- fibroadenomas
- lobular carcinoma in situ

Tightly cohesive three-dimensional aggregates:

- fibroadenomas and phyllodes tumors
- intraductal papilloma
- lobular carcinoma in situ
- ductal proliferative lesions from intraductal hyperplasia to ductal carcinoma in situ
- well-differentiated ductal carcinomas
- mucinous carcinomas

Loosely cohesive three-dimensional clusters:

- phyllodes tumor
- ductal carcinoma in situ

Branching papillary clusters:

- fibroadenoma
- intraductal papilloma
- papillary carcinoma

Numerous single cells:

- carcinoma
- pregnancy or lactation
- non-Hodgkin lymphoma
- intramammary lymph node

Background elements include inflammatory cells, amorphous debris, fresh and old blood, and mucin. Acute inflammatory cells are seen with mastitis and necrotic carcinomas. Chronic inflammatory cells are noted with (hyperplastic) intramammary lymph nodes, medullary carcinoma, and lymphoproliferative disorders. Although amorphous granular debris suggests malignancy, it does not provide sufficient grounds for making a positive diagnosis because it may also be observed with pregnancy or lactation and apocrine metaplasia. Blood is a clue to an intraductal papilloma, papillary or another carcinoma, and angiosarcoma. Mucin or myxoid material is seen with a fibroadenoma, mucinous carcinoma, or a mucocele.

High-power examination:

- types of isolated cells
- nuclear characteristics
- cytoplasmic characteristics

Isolated cells are epithelial or mesenchymal in origin and may be intact or stripped of cytoplasm (naked nuclei). Isolated epithelial cells are seen in pregnancy or lactation, and carcinoma. Mesenchymal cells are noted in fibroadenoma, phyllodes tumor, invasive carcinoma, and sarcoma. Naked nuclei are common in pregnancy or lactation and fibroadenoma. Inflammatory cells are commonly seen in fat necrosis, FCC, mastitis, lymphoma, and intramammary lymph nodes. Histiocytes are seen in fat necrosis, radiation, FCC, granulomas, and status postsilicone injection or ruptured silicone implant.

Nuclear atypia is assessed based on nuclear location, size, and shape; the chromatin pattern; and the quality of nucleoli. Although the standard cytologic criteria for malignancy (eccentric, large, angulated, pleomorphic nuclei with irregular and large nucleoli) apply to moderately and poorly differentiated ductal carcinomas, some malignant tumors, including tubular, lobular, and mucinous carcinoma, show little nuclear atypia. The recognition of other features, such as the architectural arrangement or the presence of abundant extracellular mucin, is important in the diagnosis of these tumors.

Cytoplasmic characteristics are useful in classifying some breast lesions. Distinctive features include apocrine change and cytoplasmic vacuolization. Apocrine cytoplasm is seen in apocrine metaplasia and apocrine carcinoma. Vacuolated cytoplasm is observed with pregnancy or lactation, fat necrosis, radiation effect, mucinous carcinoma, lobular carcinoma, lipid-rich carcinoma, secretory carcinoma, and status postsilicone injection or ruptured silicone implant.

THE NORMAL BREAST

The breast contains 15 to 25 lactiferous ducts, which begin at the nipple, then branch into smaller ducts, and end in the terminal duct lobular unit (or lobule). The lobule is composed of a terminal duct and many small ductules (or acini). An inner layer of cuboidal or columnar epithelial cells and an outer layer of myoepithelial cells line all ducts and ductules. The connective tissue within the lobule is a hormonally responsive mixture of fibroblasts, occasional lymphocytes, and histiocytes, in a background of collagen and acid mucins. The interlobular stroma is hypocellular and contains fibroadipose tissue. During pregnancy there is a marked proliferation of ductules, which results in large lobules, and the epithelial cells have abundant cytoplasm filled with secretory vacuoles. These secretory changes usu-

ally disappear after lactation; in some cases, however, they persist for years after pregnancy and are sometimes seen even in patients who have not been pregnant. The cause in such cases has been ascribed to pharmaceutical agents.

BENIGN CONDITIONS

Fibrocystic Changes

FCC is the most common breast disorder. It is characterized by any combination of small or large cysts, apocrine metaplasia, focal fibrosis, adenosis, and intraductal hyperplasia. These changes can result in palpable, sometimes painful, masses. Cysts arise from the terminal duct lobular units by an unfolding and simplification of adjacent ductules. Moderate and florid ductal hyperplasia, which is seen in some but not all cases, is a marker for increased cancer risk. For this reason, FCC is usually categorized as non-proliferative or proliferative, depending on whether significant intraductal hyperplasia is present.

Nonproliferative Fibrocystic Changes

CYTOMORPHOLOGY OF NONPROLIFERATIVE FIBROCYSTIC CHANGES:

- apocrine cells
- foam cells
- small ductal cells

Nonproliferative lesions yield a scant specimen when the lesion is predominantly fibrous. When a cyst is present, the specimen is a fluid that may be thin and yellow or thick and darker in color. Apocrine cells line many but not all benign cysts. Apocrine cells have abundant granular cytoplasm that stains pink or green with the Papanicolaou stain and gray with a Romanowsky stain (Fig. 8.4). Nuclei are centrally located, round, and regular, with prominent eosinophilic nucleoli, and moderate nuclear atypia is present in some cases. Benign apocrine cells are arranged as flat sheets; isolated cells are rare. Foam cells have abundant cytoplasm that is vacuolated rather than granular. Ductal epithelial cells are arranged in sheets and three-dimensional clusters (Fig. 8.5).

DIFFERENTIAL DIAGNOSIS OF NON-PROLIFERATIVE FIBROCYSTIC CHANGES:

- granular cell tumor
- apocrine carcinoma

Granular cell tumors of the breast are rare. The cells of this tumor, thought to be of Schwann cell origin, have a low nuclear-to-cytoplasmic ratio, a small nucleus, and a coarsely granular cytoplasm.¹²⁹ The background

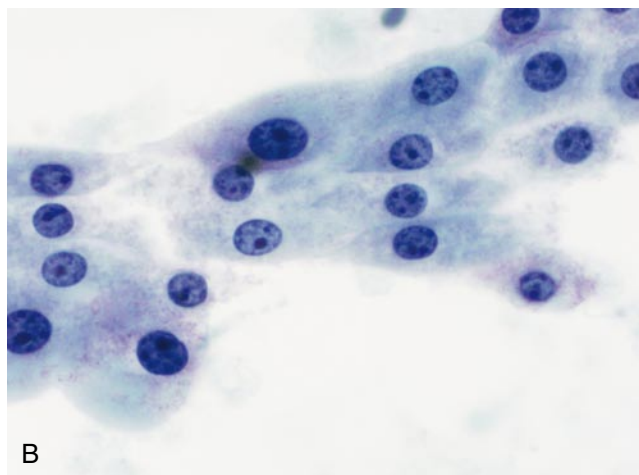
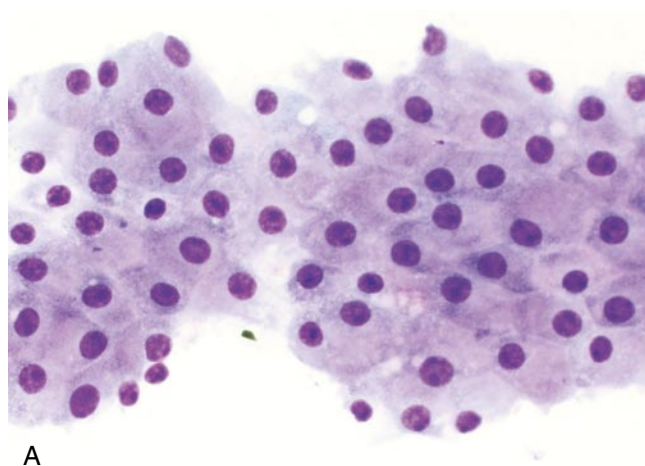


Figure 8.4 Apocrine metaplasia. Large, flat sheets of apocrine cells have distinct cytoplasmic borders, a centrally located nucleus, and a prominent nucleolus. Abundant granular cytoplasm is gray-purple on Diff-Quik stain A, and green on Papanicolaou stain B.

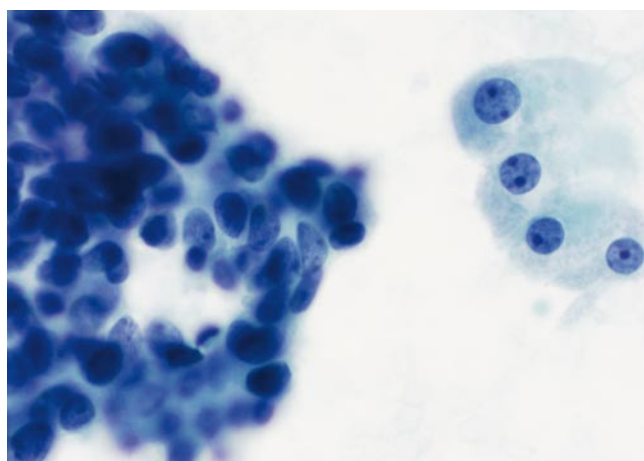


Figure 8.5 Benign ductal epithelium in nonproliferative fibrocystic changes (FCC). A tightly cohesive cluster of ductal epithelial cells without atypia is noted adjacent to apocrine metaplastic cells (Papanicolaou stain).

is usually clean. An apocrine carcinoma should be suspected when there is hypercellularity, marked nuclear atypia, and pronounced cell dyshesion, but a cautious diagnostic approach is advisable, because marked variation in nuclear size is also seen in some benign cysts. Apocrine adenosis, although rare, can also present with nuclear atypia, but there is less nuclear hyperchromasia than with carcinoma, and there are many naked nuclei.¹³⁰

Proliferative Fibrocystic Changes

Ductal proliferative lesions (proliferative FCC) comprise a group of lesions that vary in their severity and degree of atypia. The spectrum includes proliferative lesions without atypia (“usual ductal hyperplasia”), atypical ductal hyperplasia, and atypical lobular hyperplasia. The criteria that define these entities and distinguish them from carcinoma in situ are histologic, not cytologic.¹³¹ Nevertheless, the degree of crowding and nuclear atypia allows for separation of the higher end of this spectrum from the lower. FNAs diagnosed as atypical should probably be excised because the morphologic features of well-differentiated in situ and invasive carcinomas overlap with those of benign entities.¹²⁸

CYTOMORPHOLOGY OF DUCTAL PROLIFERATIVE LESION WITHOUT ATYPIA:

- sheets and tight clusters of cells without significant nuclear overlap
- regular cellular spacing
- finely granular chromatin pattern
- inconspicuous to small nucleus
- Figure 8.6

CYTOMORPHOLOGY OF DUCTAL PROLIFERATIVE LESION WITH ATYPIA:

- sheets and tight clusters of cells with significant nuclear overlap
- regular to irregular cellular spacing
- finely to coarsely granular chromatin
- prominent to multiple nucleoli
- Figures 8.7 and 8.8

DIFFERENTIAL DIAGNOSIS OF DUCTAL PROLIFERATIVE LESION WITHOUT ATYPIA:

- intraductal papilloma
- fibroadenoma
- carcinoma in situ

An intraductal papilloma is cytologically indistinguishable from proliferative FCC. Unlike proliferative FCC, however, many intraductal papillomas present as a nipple discharge or a discrete subareolar mass. Proliferative FCC may be impossible to distinguish from

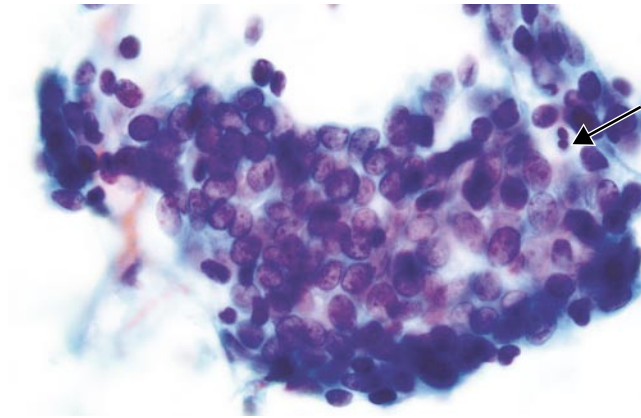


Figure 8.6 Ductal proliferative process. Note the interspersed myoepithelial cells, which stand out like sesame seeds on a bun (Papanicolaou stain).

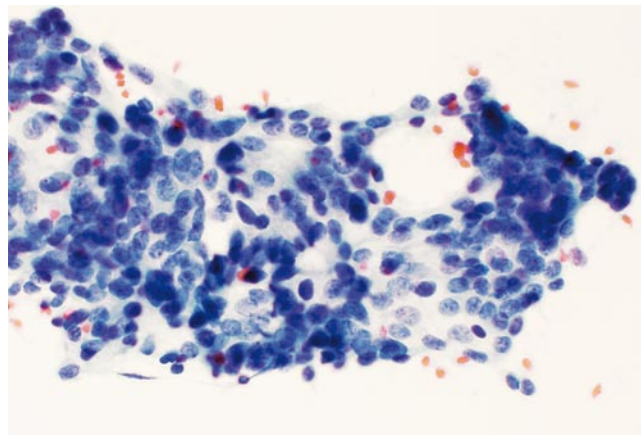


Figure 8.7 Atypical ductal proliferative process (atypical ductal hyperplasia bordering non-comedo ductal carcinoma in situ [DCIS]). In contrast to Figure 8.6, there is less regular nuclear spacing and more prominent nuclear atypicity. Such proliferative lesions cannot be categorized precisely by fine-needle aspiration (FNA), Papanicolaou stain).

a fibroadenoma or phyllodes tumor,¹³² except that stromal fragments are fairly common in the latter two, and rarely seen in proliferative FCC.

Pleomorphic carcinoma cells, calcium, necrosis, large nucleoli, and macrophages are indicative of comedo-type ductal carcinoma in situ and are usually diagnosed as suspicious or positive (see Fig. 8.8).^{133,134} Cases of low- or intermediate-grade ductal carcinoma in situ (DCIS) are usually placed into the atypical category.¹¹⁴ Some but not all well-differentiated DCIS lesions show more dyshesion than does proliferative FCC. Still, distinguishing florid ductal hyperplasia, atypical ductal hyperplasia, and well-differentiated DCIS from one another is difficult,^{88,119,128,134-138} even with the aid of image cytometry^{139,140} Although intraductal carcinomas are more likely than intraductal papillomas or other benign intraductal lesions to have numerical aberrations of chromosomes detected using fluorescence in situ hybridization (FISH)

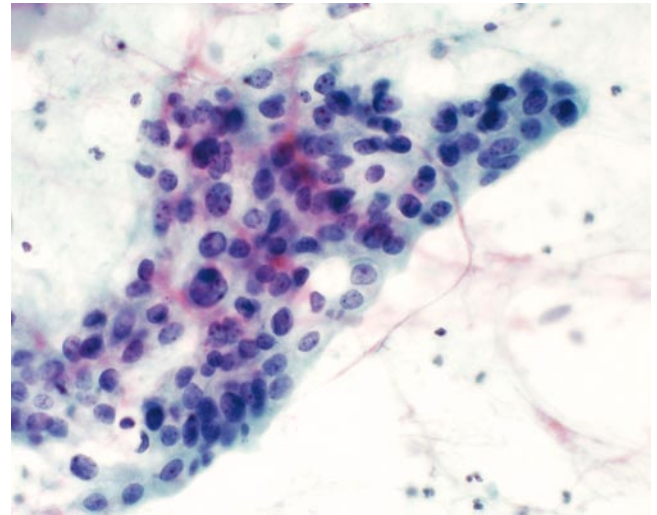


Figure 8.8 Suspicious for malignancy (comedo-type ductal carcinoma in situ [DCIS]). The cells are loosely cohesive with marked nuclear pleomorphism, nucleoli, and a dirty background. Such specimens cannot be distinguished from invasive carcinoma (Papanicolaou stain).

on FNA specimens,¹⁴¹ it is unlikely that ductal proliferative lesions will be easily categorized in the near future. This is not surprising because primarily architectural, not nuclear or cellular, features define these lesions. The use of cell blocks has been proposed as an adjunct to help in more precise cytologic classification of these entities.¹⁴²

Fibroadenoma

Fibroadenoma (FA) is the most common benign tumor of the female breast. Although more common in young women, it is seen in women of any age, including those who are postmenopausal. FAs are well circumscribed, freely movable, rubbery masses that result from both stromal and glandular proliferation.

CYTOMORPHOLOGY OF FIBROADENOMA:

- hypercellular
- large sheets (Fig. 8.9)
- three-dimensional clusters with an antler-like configuration (Fig. 8.10)
- bipolar cells and spindle or oval naked nuclei (Fig. 8.11)
- fibrillar stromal fragments
- bluish-gray with the Papanicolaou stain
- intensely red-purple with a Romanowsky-type stain
- nuclear atypia (Fig. 8.12)
- some loss of epithelial cohesion
- regular nuclear spacing
- finely granular chromatin pattern
- small, round nucleolus

DIFFERENTIAL DIAGNOSIS OF FIBROADENOMA:

- ductal proliferation with or without atypia
- phyllodes tumor
- ductal carcinoma

Although no single criterion distinguishes an FA from the ductal proliferations, a combination of features permits a distinction in most cases.¹⁴³ In general, FAs are more cellular. Naked nuclei, although more abundant in FAs, are seen in both conditions. Stromal fragments and

papillary antler-like configurations, seen in many (but not all) FAs, are uncommon in FCC.^{143,144}

The distinction between FA and phyllodes tumor is difficult. Numerous individual, long, plump, spindle-shaped nuclei, are characteristic of a phyllodes tumor.^{145,146} Hypercellular stromal fragments are more common in phyllodes tumor¹⁴⁶ but can be seen in FA as well.

The distinction between FA and ductal carcinoma is usually straightforward; the most helpful diagnostic features are stromal fragments, antler-like epithelial configurations, and honeycomb sheets of ductal cells, all of which are uncommon in ductal carcinomas. Some features can be misleading, however. Cytologic atypia is

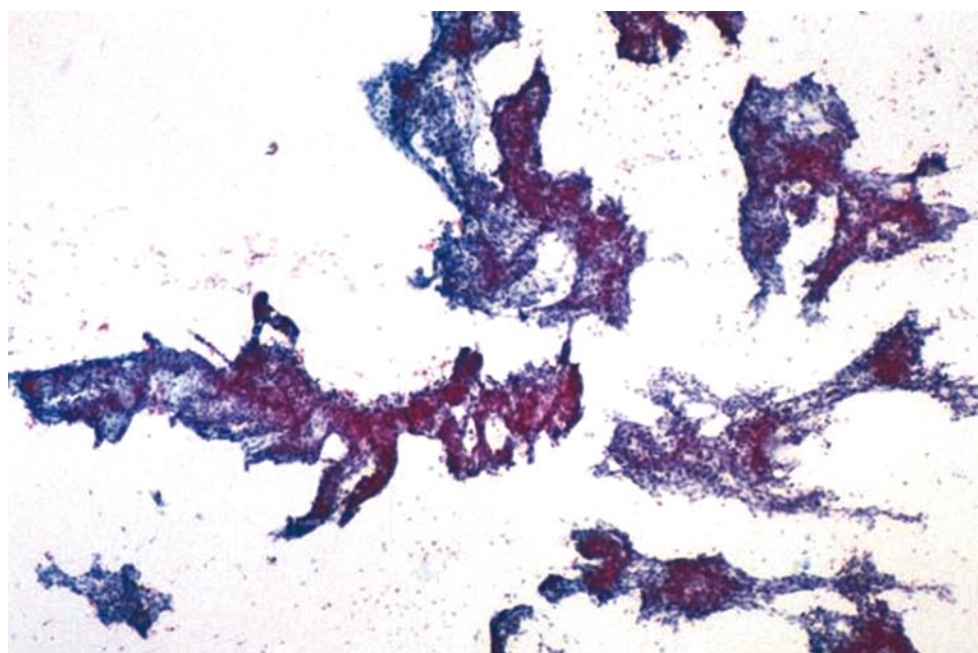


Figure 8.9 Fibroadenoma (FA), low power. The specimen is hypercellular with many folded sheets and antler-horn clusters (Papanicolaou stain).

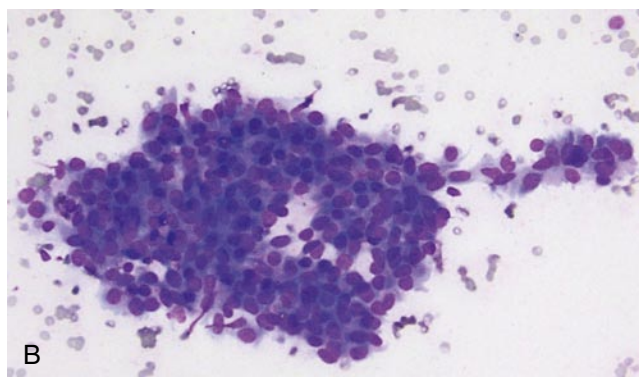
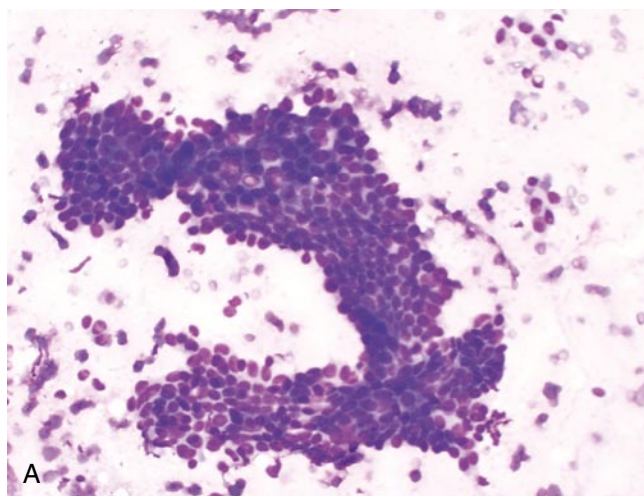


Figure 8.10 Fibroadenoma (FA). A, Branching antler-horn clusters are the predominant arrangement of cells. There are rare stripped naked nuclei and bipolar cells in the background, but they are not prominent in this smear, making it difficult to distinguish from a ductal proliferative process (Diff-Quik stain). B, Clusters of tightly cohesive cells with minimal nuclear atypicity are characteristic of fibroadenomas (Diff-Quik stain).

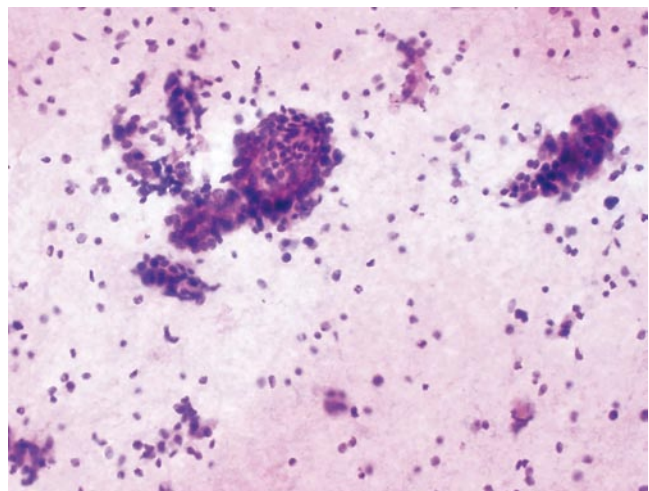


Figure 8.11 Fibroadenoma (FA). Clusters of epithelial cells in a background of numerous stripped elongated naked nuclei are characteristic of FAs. When stromal cells are absent, the diagnosis is more difficult (Papanicolaou stain).

prominent in some FAs,^{143,147-149} and isolated cells with intact cytoplasm, a highly characteristic feature of ductal carcinoma, are seen in about 20% of FAs.^{68,143,149-151} Conversely, some ductal carcinomas masquerade as FAs. The greatest mimics are well-differentiated invasive ductal carcinoma and DCIS. Naked nuclei, characteristic of FAs, are seen in some ductal carcinomas,¹⁵⁰ although usually in fewer numbers than in an FA. Nuclear hyperchromasia favors a diagnosis of malignancy, whereas nuclei with small, uniform nucleoli suggest FA.¹⁵⁰ The distinction is not discernible in all cases, and the diffe-

rential diagnosis may be difficult especially in older patients.^{143,150,151} Another mimic of FAs is papillary carcinoma.¹⁵² Most single epithelial cells on FNA from carcinomas are round to oval with eccentric nuclei, and those from FAs are elongated or columnar with cytoplasm on both sides of the nucleus. However, papillary carcinomas can present with spindle- or columnar-shaped single epithelial cells on FNA. Smears with equivocal findings should be reported as atypical or suspicious.

Pregnancy and Lactational Changes

During pregnancy and lactation, the ductules of the terminal duct lobular unit become hyperplastic and manifest cytoplasmic vacuolization and luminal secretion. Occasionally, this change results in a discrete nodule, called a lactating adenoma (LA), which is difficult to distinguish clinically from a malignancy. Because carcinoma is occasionally diagnosed in the setting of pregnancy, this diagnosis must be excluded in a pregnant or lactating woman. FNA in this setting may be especially useful because a diagnosis of pregnancy or lactational changes could at least postpone and even spare the woman an excisional biopsy.

CYTOMORPHOLOGY OF PREGNANCY AND LACTATIONAL CHANGES:

- moderately cellular specimen
- numerous isolated epithelial cells or stripped nuclei

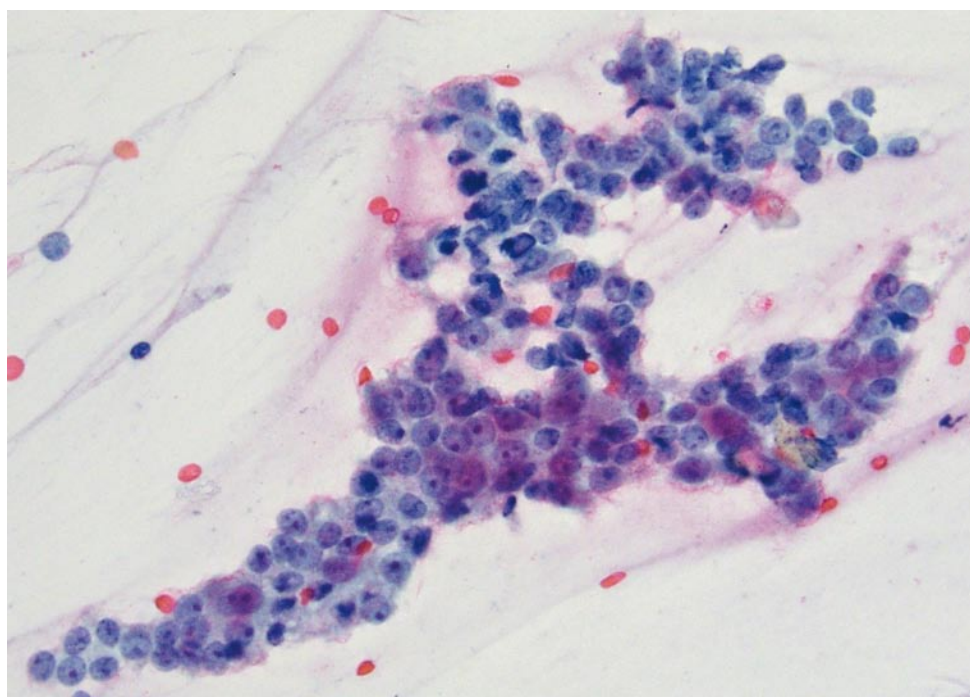


Figure 8.12 Fibroadenoma (FA). Note the prominent nuclear atypia with nucleoli. The tightness of the cluster and a background of single bipolar cells and stripped nuclei are important clues in avoiding an overdiagnosis of malignancy (Papanicolaou stain).

- nuclear enlargement without variation in size or shape
- prominent nucleolus
- abundant delicate and wispy granular or finely vacuolated cytoplasm
- cytoplasm easily strips away
 - foamy proteinaceous background
 - many naked nuclei
- occasional small ductal cell clusters and portions of lobules
- [Figure 8.13](#)

DIFFERENTIAL DIAGNOSIS OF PREGNANCY AND LACTATIONAL CHANGES:

- carcinoma, both lobular and ductal
- non-Hodgkin lymphoma

The cells of invasive lobular carcinoma are similar in size to those of a LA, but the foamy background, intact acini and lobules of benign breast tissue, and the prominent nucleoli of a LA are absent in invasive lobular cancers. The nuclear size and shape of LA cells can also resemble those of some well-differentiated ductal cancers, and the cytoplasmic features can overlap with secretory carcinoma.¹⁵³ In general, ductal cancers do not have the foamy background characteristic of an LA, and the cohesive groups of malignant cells in ductal cancers are not arranged in normal acinar structures. Also, the nuclei in ductal cancers are more hyperchromatic, the nucleoli less prominent, and the cytoplasm less wispy than in LA.

Non-Hodgkin lymphoma can be confused with pregnancy or lactation. Both have many single cells with prominent nucleoli, but the cytoplasmic features, pro-

teinaceous background, and intact benign breast tissue typical of LAs are helpful. The isolated cells of lymphoma vary more in size and shape than those of an LA.¹⁵⁴

Fat Necrosis

Fat necrosis (FN) can mimic carcinoma both clinically and mammographically and is commonly seen in patients who have had a previous surgical biopsy or other trauma to the breast. FN is also encountered in male patients.¹⁵⁵

CYTOMORPHOLOGY OF FAT NECROSIS:

- hypocellular smears, predominantly histiocytes with fine to coarse cytoplasmic vacuoles
- round to kidney-bean shaped nucleus
- low nuclear-to-cytoplasmic ratio
- multinucleated and atypical cells
- background of neutrophils, lymphocytes, and plasma cells
- [Figure 8.14](#)

DIFFERENTIAL DIAGNOSIS OF FAT NECROSIS:

- silicone granuloma
- infections
- ductal carcinoma
- lipid-rich carcinoma

The histiocytes seen in reactions to silicone injection or a ruptured silicone implant contain vacuoles that are larger than those seen in FN and often have a signet-ring appearance.^{156,157} Histiocytes with large cytoplasmic vacuoles containing refractile budding yeast forms are

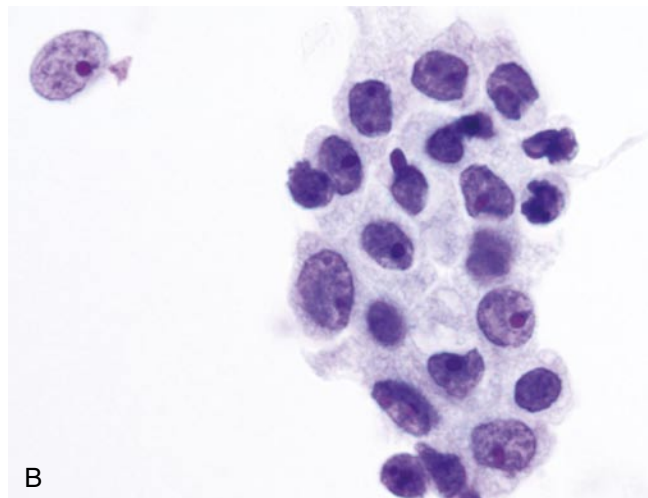
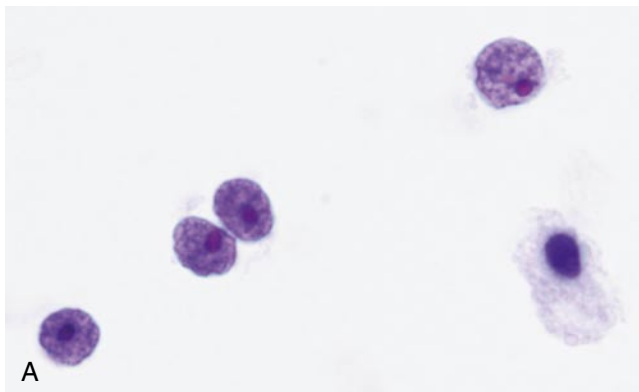


Figure 8.13 Pregnancy or lactation. A, Numerous stripped or “naked” nuclei are seen. B, Cells in loose clusters may also be seen. Nuclei are round to oval and regular with prominent nucleoli (ThinPrep, Papanicolaou stain).

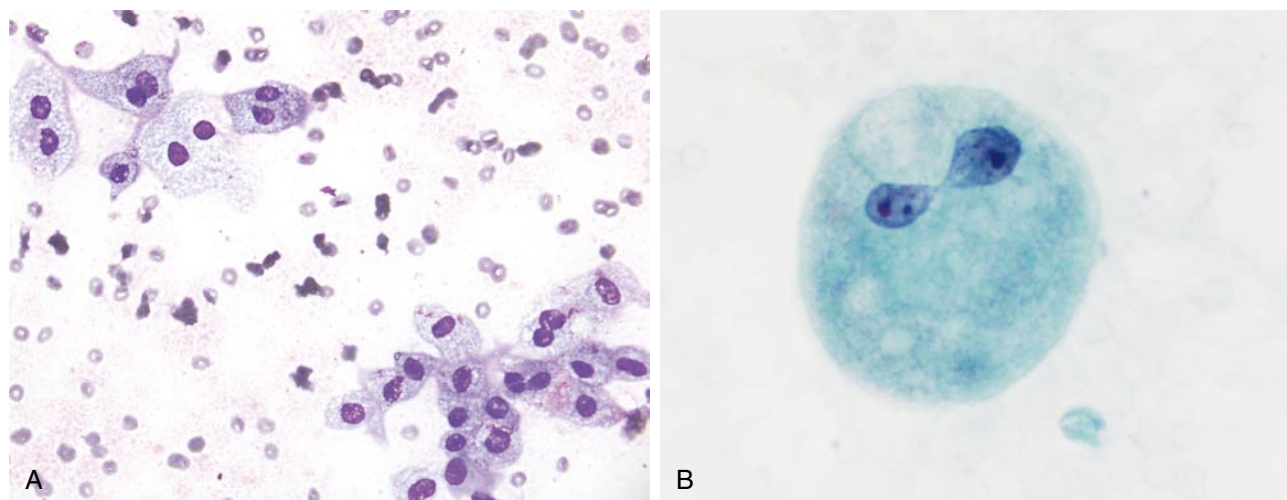


Figure 8.14 Fat necrosis (FN). A, Histiocytes with foamy vacuolated cytoplasm and oval nuclei are present (Diff-Quik stain). B, There is a single mononucleated histiocyte with a kidney-bean-shaped nucleus and vacuolated cytoplasm. Smears of FN are typically hypocellular (Papanicolaou stain).

present in cryptococcosis of the breast, which can occur in a patient who is immunosuppressed.

Some ductal carcinomas coexist with FN; they are identified by the presence of a distinct population of malignant cells in addition to histiocytes. The rare lipid-rich carcinoma can also be confused with FN because the tumor cells have abundant vacuolated cytoplasm. Unlike FN, however, a lipid-rich carcinoma is hypercellular and shows marked nuclear atypia.

Radiation Change

Radiation change in normal breast epithelium is observed with increasing frequency because of the widespread use of lumpectomy and radiation to treat patients with breast cancer. Radiation change is often seen in conjunction with FN.

CYTOMORPHOLOGY OF RADIATION CHANGE:

- hypocellular aspirate
- proportionate nuclear and cellular enlargement with low nuclear-to-cytoplasmic ratio
- hyperchromatic nuclei with a round, regular outline and prominent nucleoli
- coarse cytoplasmic vacuoles, some containing inflammatory cells
- binucleation and multinucleation
- [Figure 8.15](#)

DIFFERENTIAL DIAGNOSIS OF RADIATION CHANGE:

- FN
- recurrent carcinoma ([Fig. 8.16](#))

The histiocytic nuclei of FN are smaller than those of epithelial cells altered by radiation. Postsurgical and radiation changes are a recognized pitfall but can usually be distinguished reliably from breast cancer.¹⁵⁸⁻¹⁶¹ Most recurrent or persistent carcinomas demonstrate a moderately cellular or hypercellular smear with many atypical cells. Because radiation changes also contain highly atypical epithelial cells, however, comparison with the morphology of the original tumor is recommended. Histologic confirmation may be necessary when the cytologic diagnosis is equivocal. The absence of isolated cells and necrotic cell debris is a useful finding because these are more commonly seen in carcinomas.

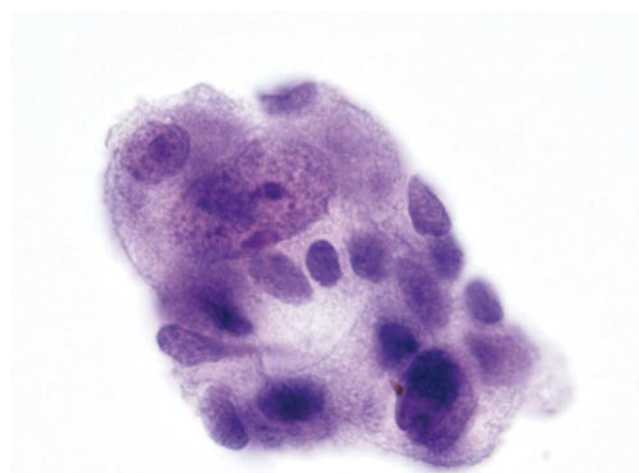


Figure 8.15 Radiation change. The cells demonstrate pronounced nuclear enlargement but also concomitant cytomegaly. The nuclear to cytoplasmic ratio is maintained (ThinPrep stain).

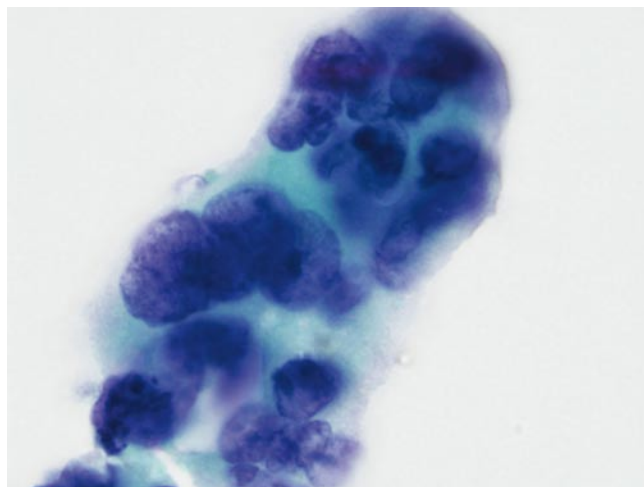


Figure 8.16 Recurrent carcinoma in a background of radiation change. In contrast to Figure 8.15, the nuclei are irregular and the nuclear-to-cytoplasmic ratio is increased (ThinPrep Papanicolaou stain).

Mastitis

Acute mastitis is usually a bacterial infection and is seen most commonly in the postpartum period. Bacteria invade the breast through the small erosions in the nipple of a lactating woman, and an abscess can result. *Chronic mastitis* can be a sequela of acute mastitis, or more commonly, associated with duct ectasia. Chronic mastitis is a disease of unknown etiology that results in the dilatation of large-sized and intermediate-sized ducts with a surrounding inflammatory infiltrate of lymphocytes and plasma cells. Some patients have a palpable mass that mimics carcinoma. A variant of chronic mastitis, characterized by an infiltrate composed predominantly of plasma cells, is called *plasma cell mastitis*. *Granulomatous mastitis* has the usual cytologic picture of granulomas and can be infectious (i.e., tubercular or fungal) in origin.¹⁶²⁻¹⁶⁴ The term *granulomatous lobular mastitis* has been given to a distinct clinical syndrome of unknown etiology that affects women months to years after pregnancy. These lesions, which present as firm masses in the periphery of the breast, can be large and may suggest malignancy. The aspirate may contain cohesive clusters of histiocytes with nuclei shaped like a “kidney bean” or a “boomerang.” Other chronic inflammatory cells such as lymphocytes and plasma cells may be prominent. Special stains for bacteria, fungi, and acid-fast organisms are mandatory to rule out infection.

CYTOMORPHOLOGY:

Acute Mastitis

- abundant neutrophils
- occasional groups of reactive ductal cells with enlarged nuclei and prominent nucleoli

Chronic Mastitis

- abundant, amorphous, granular debris from inspissated ducts
- inflammatory infiltrate composed of lymphocytes and plasma cells

Granulomatous Mastitis

- clustered epithelioid histiocytes
- abundant vacuolated cytoplasm
- round or folded nuclei
- dispersed chromatin texture
- large nucleoli
- giant cells, lymphocytes, plasma cells, and eosinophils
- rare clusters of benign ductal cells

Subareolar Abscess

Often called “recurring subareolar abscess,” this inflammatory condition arises in the areola as a result of squamous metaplasia of lactiferous ducts, with subsequent keratin plugging, dilatation, and rupture of the ducts. Without complete excision, the lesion can recur and result in the formation of sinus tracts.

CYTOMORPHOLOGY OF SUBAREOLAR ABSCESS:¹⁶⁵

- numerous anucleate squames admixed with neutrophils
- histiocytes and multinucleated giant cells
- occasional groups of atypical reactive ductal cells
- fragments of granulation tissue

Gynecomastia

Gynecomastia is the most common abnormality of the male breast. It is an enlargement of the breast that can be diffuse or nodular and is frequently bilateral.

CYTOMORPHOLOGY OF GYNECOMASTIA:¹⁶⁶

- resembles FA (Fig. 8.17)
- low, moderate, or rarely high cellularity
- groups of ductal cells with small oval nuclei, scant cytoplasm, and little variation in size and shape
- isolated bipolar cells
- naked nuclei

DIFFERENTIAL DIAGNOSIS OF GYNECOMASTIA:

- carcinoma

FNA is useful in the diagnosis of lesions of the male breast.^{167,168} Carcinoma arising in the male breast is usually of the invasive ductal type. Other subtypes occur,

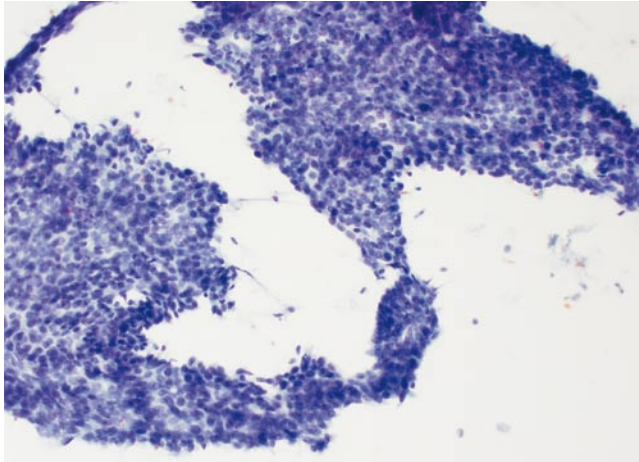


Figure 8.17 Gynecomastia. Note the cohesive flat sheets that are identical to those of fibroadenoma (FA) and ductal proliferative processes (Papanicolaou stain).

but lobular carcinoma is extremely rare.⁶⁹ The cytologic features are identical to their counterparts in women.

PAPILLARY NEOPLASMS

Intraductal papillomas (IPs) usually are solitary tumors that arise most often in the subareolar lactiferous ducts but can occur elsewhere in the breast. Because most patients present with a discharge (often bloody) from the nipple, nipple discharge cytology is the customary method of evaluation. Less commonly, a patient presents with a subareolar mass that is evaluated by FNA.

Papillary carcinoma (PC) represents 1% to 2% of breast carcinomas. It is defined as a malignant tumor in which the predominant growth pattern is frondlike. It may be invasive or noninvasive, cystic or solid, and is associated with a favorable prognosis. About one-half of PCs arise in the central portion of the breast. It is therefore not surprising that up to one third of patients with this tumor have a discharge from the nipple.⁶⁹

The distinction between IP and PC is virtually impossible to establish by FNA. Instead of a “positive” or “negative” diagnosis, it is best to diagnose a “papillary lesion” and then specify if possible, “favor benign/malignant.” PCs generally show a monomorphous population of abundant isolated columnar cells with intact cytoplasm. Papillary clusters of cells and tall, columnar cells in a hemorrhagic background should raise the suspicion of malignancy.¹⁶⁹ Although a pure IP is less dyshesive and less monomorphic than a PC,³⁴ some IPs have foci of intraductal PC. Sclerosing papillary lesions may present as suspicious lesions on FNA.¹⁷⁰ It is best, therefore, to recommend an excisional biopsy when a breast mass has a papillary appearance on FNA. Papillary lesions can also present difficulty on core-needle biopsy.¹⁷¹

CYTOMORPHOLOGY OF PAPILLARY NEOPLASM, FAVOR BENIGN:

- moderate to high cellularity
- three-dimensional papillary groups with fibrovascular cores or flat sheets with myoepithelial cells
- only rare isolated cells with intact cytoplasm
- polymorphic small, cuboidal or columnar cells
- round to oval nucleus with finely granular chromatin
- nucleolus may be present
- foam cells, apocrine metaplasia, and inflammation may be present
- [Figure 8.18](#)

CYTOMORPHOLOGY OF PAPILLARY NEOPLASM, FAVOR MALIGNANT:

- moderate to marked cellularity
- papillary clusters and cribriform or tubular architecture with absence or paucity of myoepithelial cells
- numerous isolated cells
- often uniform tall and columnar cells
- elongated uniform nuclei
- many naked nuclei but no bipolar cells
- blood- and hemosiderin-laden macrophages common
- [Figure 8.19](#)

DIFFERENTIAL DIAGNOSIS OF A PAPILLARY NEOPLASM:

- intraductal hyperplasia
- FA

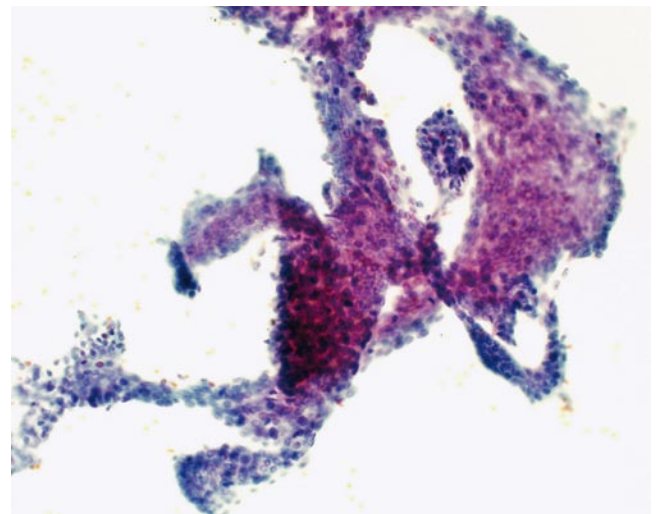


Figure 8.18 Papillary neoplasm. An excisional biopsy showed that this lesion was an intraductal papilloma. Note the complex branching structure (Papanicolaou stain).

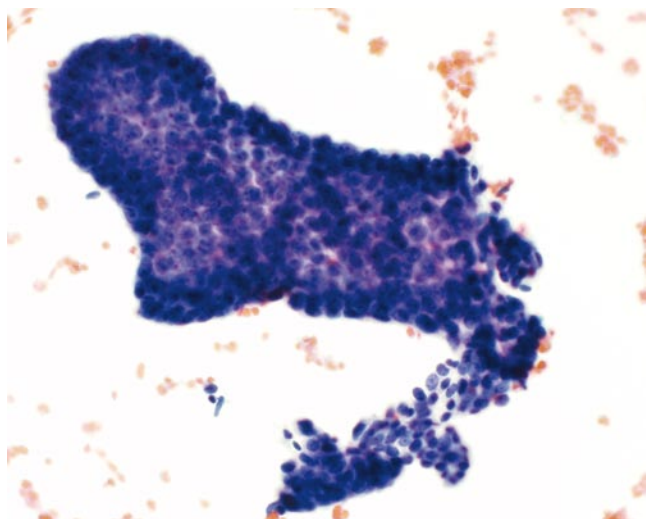


Figure 8.19 Papillary neoplasm. In contrast to Figure 8.18, this lesion proved to be a papillary carcinoma. They are similar cytologically and best diagnosed as papillary neoplasms with the definitive diagnosis deferred to an excisional biopsy (Papanicolaou stain).

The architectural pattern, presence and quantity of myoepithelial cells, and bipolar naked nuclei are useful features in distinguishing among these lesions.^{152,172} Although intraductal hyperplasia is cytologically indistinguishable from an IP, it is rare for a patient to present with a discharge from the nipple or with a subareolar mass. These cases usually show two-dimensional arrangements with myoepithelial cells.

The similarity of PC to an FA can be striking.¹⁵² Hemorrhage, a common feature of PCs, is quite uncommon in FAs. In PC, the frondlike clusters are composed of considerably dyshesive tumor cells that have a tendency

to fall away from adjacent cells. FA more often presents with folded branching clusters. There is little dyshesion, but moderate to numerous bipolar nuclei often form doublets in the background. Prominent dyshesion only rarely occur in FAs. A cellular stroma can also be seen.

PHYLLODES TUMOR

Like FAs, phyllodes tumors (PTs) are biphasic, composed of an epithelial and a stromal proliferation but with more pronounced stromal cellularity. PTs are much less common than FAs, however, accounting for less than 1% of all breast tumors. Often growing to massive proportions, they can mimic carcinoma by distorting the breast and even ulcerating the overlying skin. PTs can be benign, borderline, or malignant,¹⁷³ but the distinction cannot be made on FNA. The use of cell blocks has been suggested to be helpful in this diagnosis.¹⁴² Although stromal hypercellularity and a markedly increased stromal to epithelial ratio favors malignancy,¹⁷⁴ histologic confirmation is necessary inasmuch as an invasive margin, among other features, is important in evaluating malignant potential.⁶⁹

CYTOMORPHOLOGY OF PHYLLODES TUMOR:

- similar to FA (Fig. 8.20) but more cellular (Fig. 8.21) with a more cellular stromal component (see Fig. 8.21)
- sometimes marked stromal atypia with numerous mitotic figures (Fig. 8.22)
- pronounced epithelial atypia mimicking carcinoma

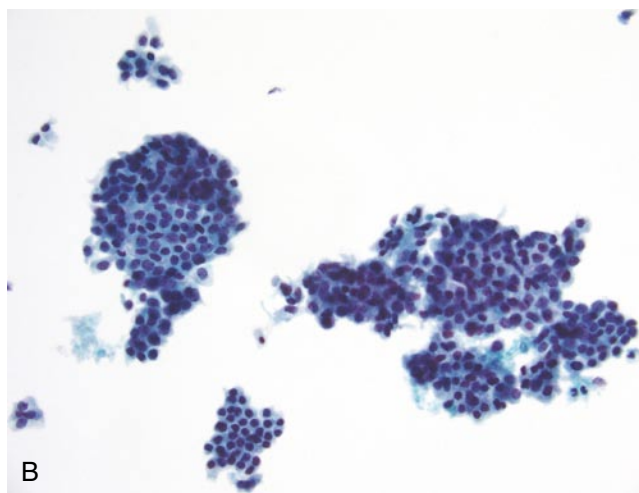
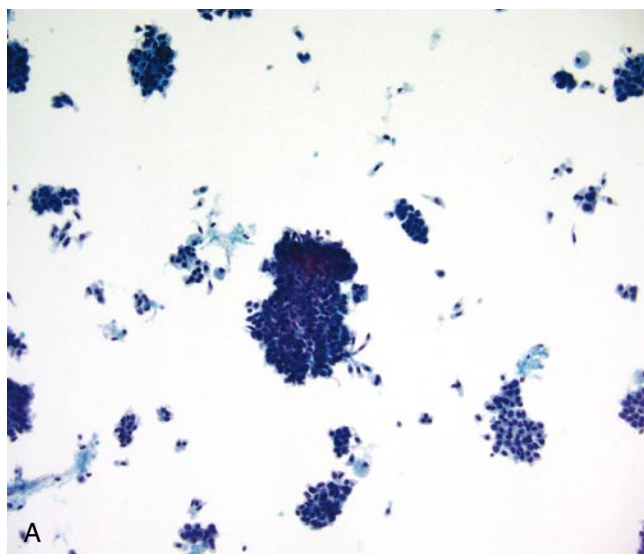


Figure 8.20 Phyllodes tumor. A, Similar cytologically to a fibroadenoma (FA), a phyllodes tumor (PT) has greater cellularity. B, The difference between stromal and epithelial cells is subtle and can be lost in a liquid-based preparation (ThinPrep, Papanicolaou stain).

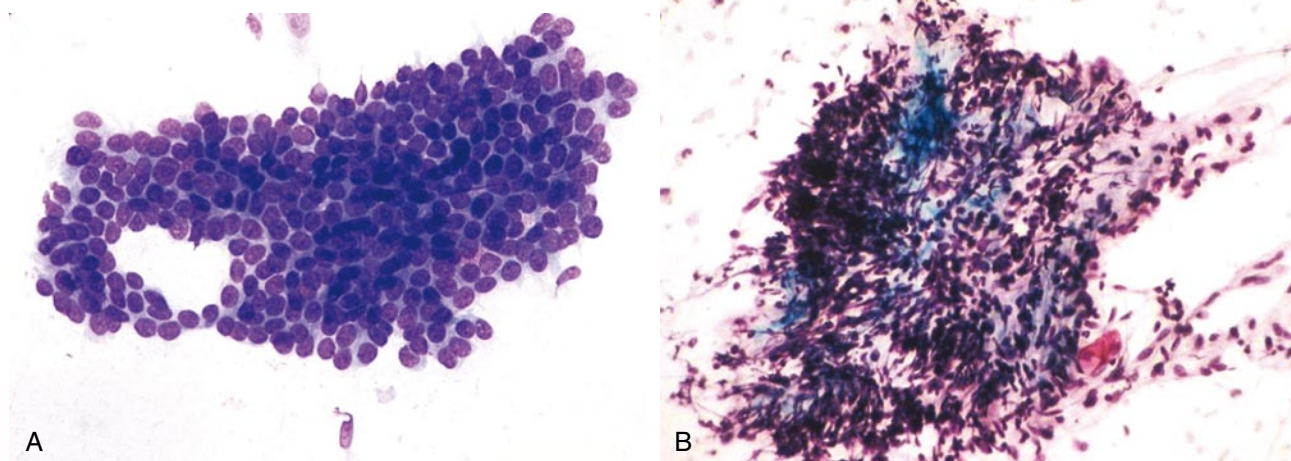


Figure 8.21 Phyllodes tumor (PT). A, Epithelial clusters in a phyllodes tumor resemble those of fibroadenoma (FA) but may be more crowded (Diff-Quik stain). B, Stromal clusters may be cellular (Papanicolaou stain).

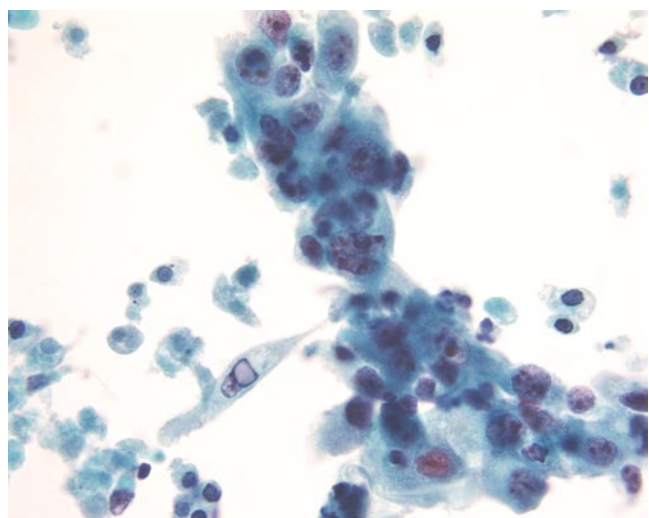


Figure 8.22 Phyllodes tumor (PT). Atypical stromal and epithelial cells are noted. The background of this tumor is necrotic. Although cytologically more alarming than the previous case (see Fig. 8.21), this tumor was a low-grade PT and the one in Figure 8.21 was a high-grade tumor (ThinPrep, Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF PHYLLODES TUMOR:

- FA
- ductal carcinoma
- metaplastic carcinoma
- primary sarcoma of the breast

In contrast to FA, the stromal fragments of a PT are more cellular and contain larger and more atypical spindle cells, particularly dispersed cells and long, spindled nuclei.^{145,175-178} A definite distinction is not possible in all

cases, and core or excisional biopsy may be necessary.¹⁷⁹ Because epithelial atypia can be pronounced, some PTs are mistaken for ductal carcinomas and a significant cause of false-positive diagnoses.^{138,175,180-182} The presence of stromal fragments and some benign ductal cells supports a diagnosis of PT.¹⁸³ Metaplastic carcinoma can mimic a PT because of a prominent spindle cell component,¹⁸⁴ but the benign epithelial component of a PT is absent.

BREAST CANCER

In the United States, breast cancer causes 20% of all deaths from cancer in women.¹⁸⁵

Invasive Ductal Carcinoma

Invasive ductal carcinoma (IDC) is the most common malignant tumor of the breast, accounting for 40% to 75% of all breast cancers.¹⁷³ IDC is almost invariably solid and can be detected by palpation or mammography. Many IDCs have a characteristically gritty consistency, appreciable during FNA. Although most are pure ductal carcinomas, limited foci of tubular, papillary, mucinous, or medullary differentiation can be present. IDC ranges from well to poorly differentiated, and is usually graded by a combination of nuclear and architectural features; thus FNA is of limited use on grading breast carcinomas.^{186,187} A subtype of IDC known as scirrhous carcinoma is characterized by abundant, dense fibrosis. Because of fibrosis, scirrhous carcinomas may result in nondiagnostic FNA specimens despite multiple passes.¹⁸⁸ In this circumstance a tissue biopsy is needed for diagnosis.

CYTOMORPHOLOGY OF INVASIVE DUCTAL CARCINOMA:

- hypercellular (Fig. 8.23)
- isolated cells and poorly cohesive clusters of cells (Fig. 8.24)
- eccentric nucleus often protruding from the cytoplasm (i.e., “comet cells”; Fig. 8.25)
- enlarged, variably hyperchromatic nuclei but can vary considerably in size and shape (Fig. 8.26)
- finely or coarsely granular chromatin pattern
- small or large and prominent, and irregularly shaped nucleolus
- usually clean background but can see inflammation, blood, and granular debris (Fig. 8.27)

DIFFERENTIAL DIAGNOSIS OF INVASIVE DUCTAL CARCINOMA:

- DCIS
- FA or PT
- proliferative FCC
- pregnancy or lactational changes

The differential diagnosis includes DCIS, the presumed precursor lesion to IDC. Not surprisingly, IDC and DCIS appear identical on cytologic examination.^{137,189,190} The significance of malignant cells embedded in fat or stroma is controversial.¹⁹¹ Some authors believe that invasion can be suggested if strict criteria (e.g., identification of “true infiltration” of fibrofatty tissue) are

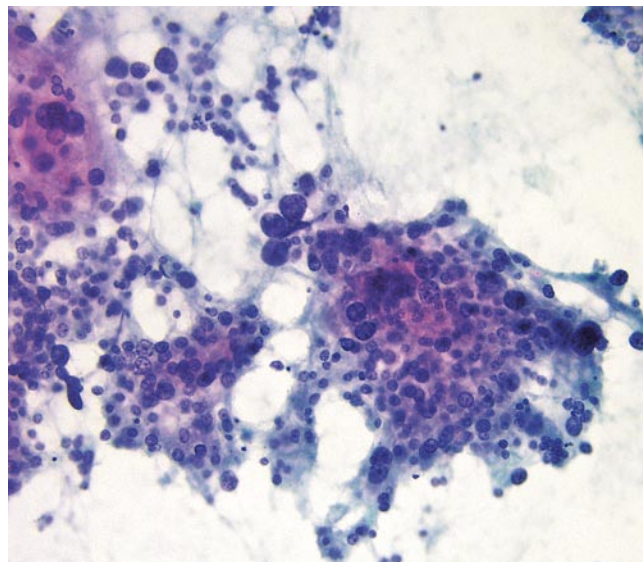


Figure 8.24 Ductal carcinoma. The specimen is cellular and the cells are present both singly and in loosely cohesive clusters (Papanicolaou stain).

applied to smears.^{133,192} Others have found this finding unreliable because it is seen in the majority of cases of DCIS.¹⁹³ In fact, benign ductal cells are commonly seen in association with fatty tissue.¹⁹³ This finding should not be taken as a sign of malignancy; rather, it is a mechanical artifact of aspiration and smear preparation. The cohesiveness of some invasive tumor cells, and the lack of tubular structures can suggest in situ carcinoma.¹⁹⁴ Important clues to the presence of invasion are cell

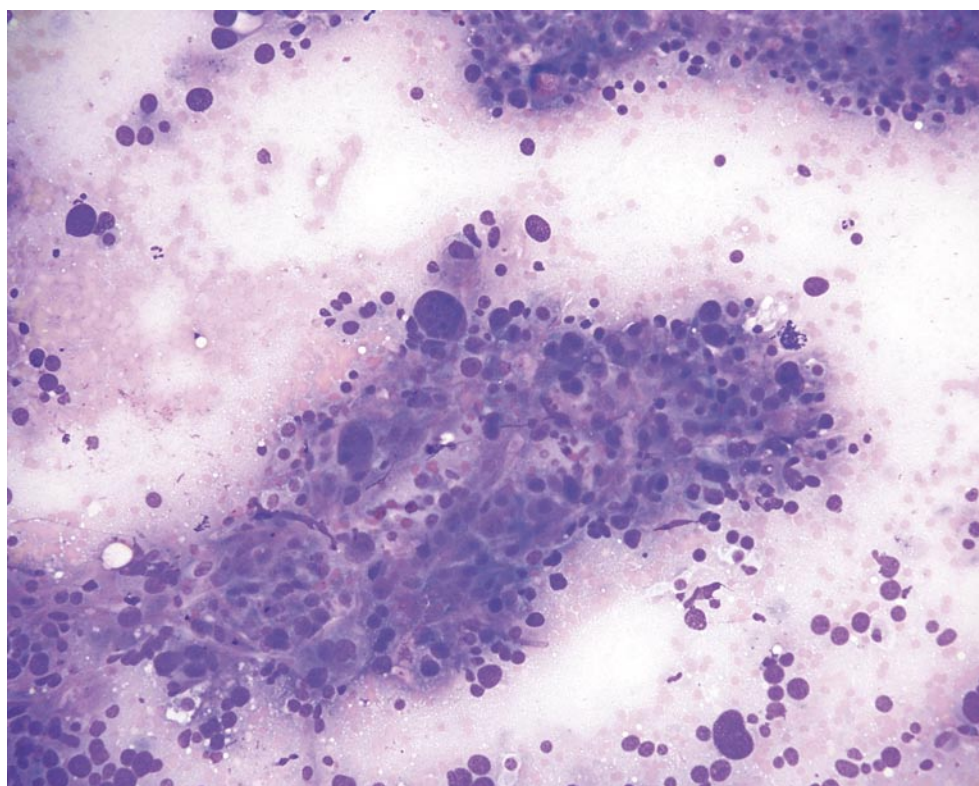


Figure 8.23 Ductal carcinoma. A major criterion for the diagnosis of ductal carcinoma is hypercellularity. Even at lower power, the nuclear atypia is also prominent (Diff-Quik stain).

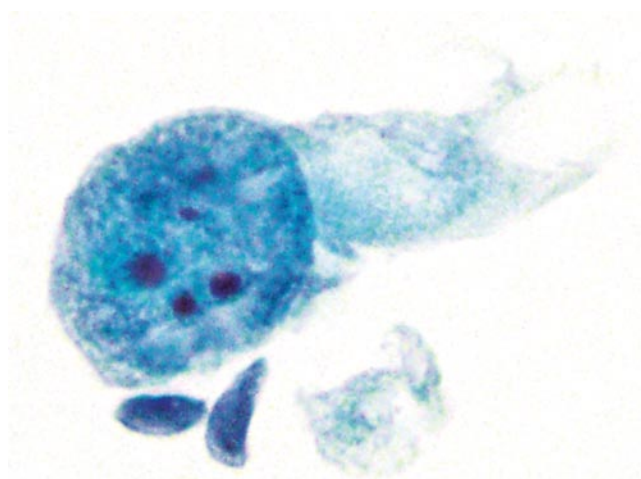


Figure 8.25 Ductal carcinoma. Many of the isolated cells of ductal cancers are comet shaped, with a nucleus that protrudes from the cytoplasm. Whether or not the nuclear atypia is marked, a protuberant nucleus suggests carcinoma (ThinPrep Papanicolaou stain).

clusters with a tubular structure, cytoplasmic lumen formation in malignant cells, fibroblast proliferation, and fragments of elastoid stroma.¹⁹⁵ Although these features may be specific, their sensitivity is low (48%). Like IDC, DCIS can present as a palpable mass or a nonpalpable mammographic abnormality. Because of these inherent difficulties, it has been suggested that cell blocks can aid in the diagnosis of invasion.¹⁴²

Well-differentiated ductal carcinomas are an important cause of false-negative results and can masquerade as FAs or PTs.^{128,138,150,151,175,180,182,196,197} Even morphometric analysis is not particularly useful in this distinction.^{198,199} Because isolated cells with intact cytoplasm can be seen in both benign and malignant lesions, their presence alone is not diagnostic of malignancy. Isolated cells with nuclear atypia, however, are highly characteristic of malignancy. Nuclear

hyperchromasia suggests ductal carcinoma; nuclei with a single, small, uniform nucleolus are more typical of FAs.¹⁵⁰ Stromal fragments and bipolar cells, particularly in pairs, are more common in FAs and relatively uncommon in ductal cancers.²⁰⁰ Although marked epithelial atypia can be seen in PTs,¹⁸² stromal fragments with spindle cell atypia are not seen in ductal cancers. The use of double immunostains for cytokeratin and smooth muscle actin or p63 alone to detect myoepithelial cells can be useful in these situations.^{112,201}

Pregnancy and lactational changes may mimic carcinoma because of the presence of numerous isolated cells with prominent nucleoli. The absence of nuclear hyperchromasia, nuclear size variation, and coarse chromatin favors a benign diagnosis. Focal nuclear atypia is seen in FN, radiation change, mastitis, and subareolar abscess, but the atypia is usually mild, and other background features provide clues to the diagnosis.

Invasive Lobular Carcinoma

Invasive lobular carcinoma (ILC) constitutes 5% to 15% of all invasive breast cancers.¹⁷³ It is composed of small or medium-sized uniform cells that have a characteristic pattern of infiltration. Tumor cells usually grow in a linear, swirling fashion and often evoke a marked desmoplastic stromal reaction, although solid growth patterns are occasionally seen. The distension of cytoplasm with mucus gives some tumor cells a signet-ring shape.

CYTOMORPHOLOGY OF INVASIVE LOBULAR CARCINOMA:

- often sparsely cellular because of marked stromal fibrosis
- predominantly isolated cells with small groups or linear arrays (Fig. 8.28A)
- small- to mid-sized tumor cells

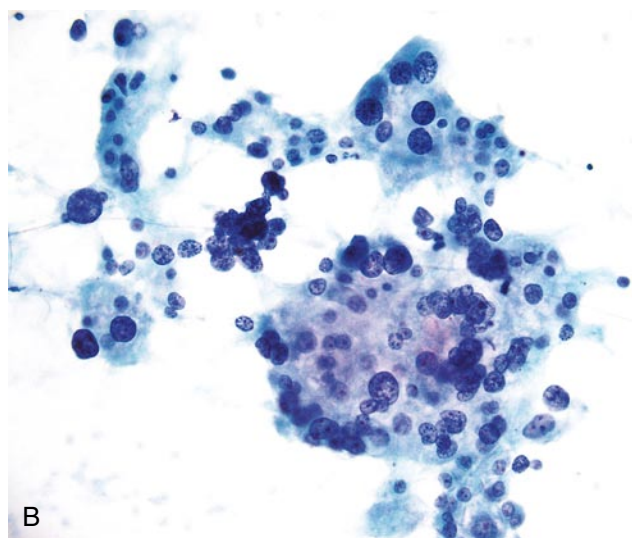
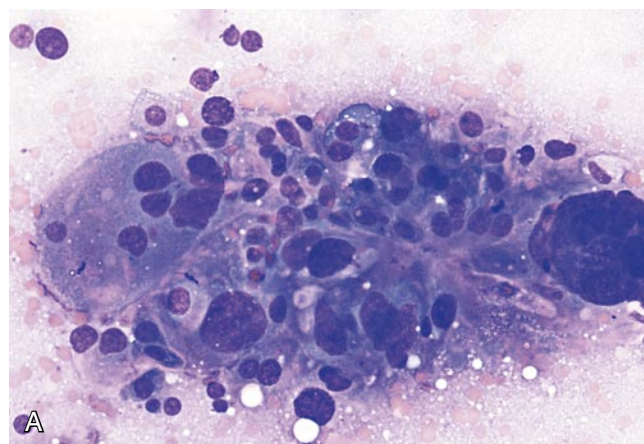


Figure 8.26 Ductal carcinoma. Note the pronounced nuclear pleomorphism, multinucleated atypical cells, and nuclear atypia with both Diff-Quik, A, and Papanicolaou, B, stains.

- large cytoplasmic vacuole (signet ring appearance) (Fig. 8.28B)
- hyperchromatic, often kidney-bean-shaped nucleus
- usually small nucleolus, but rarely large

DIFFERENTIAL DIAGNOSIS OF INVASIVE LOBULAR CARCINOMA:

- well-differentiated ductal carcinoma

This is one of the most difficult breast cancers to diagnose by FNA.²⁰² Because of the scant cellularity, most

cases are diagnosed as “atypical” or “suspicious.” The differential diagnosis includes IDC, which is usually more cellular and pleomorphic. Nevertheless, some well-differentiated IDCs are impossible to distinguish from ILC. As with IDCs, it is not possible to distinguish ILC from its precursor lesion, lobular carcinoma in situ (LCIS), by FNA. Most LCISs are moderately cellular, with cohesive clusters of cells. LCIS cells have a mildly enlarged nucleus (Fig. 8.29) and may occasionally demonstrate isolated epithelial cells, a prominent nucleolus, an intracytoplasmic lumen, and two distinct epithelial-cell populations.²⁰³ Many are diagnosed as atypical, but cases with abundant isolated cells may be overdiagnosed as ILC (Fig. 8.30).

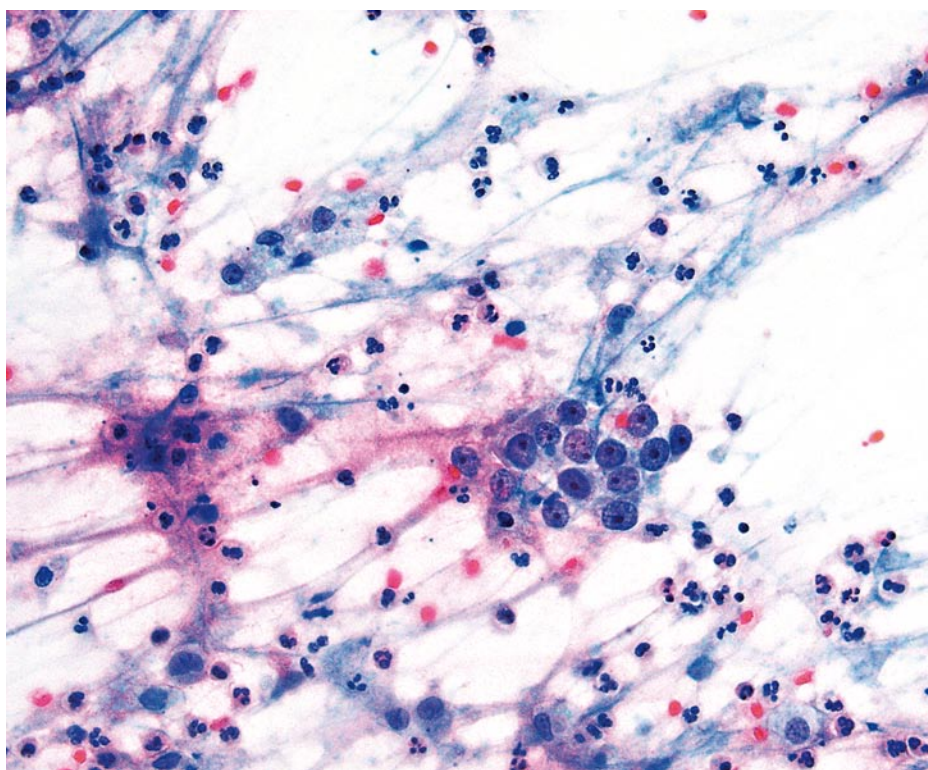
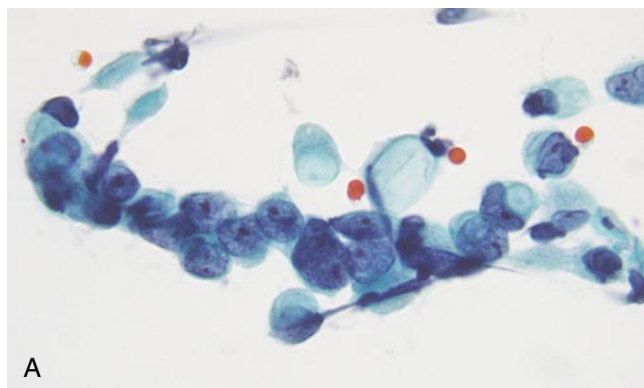
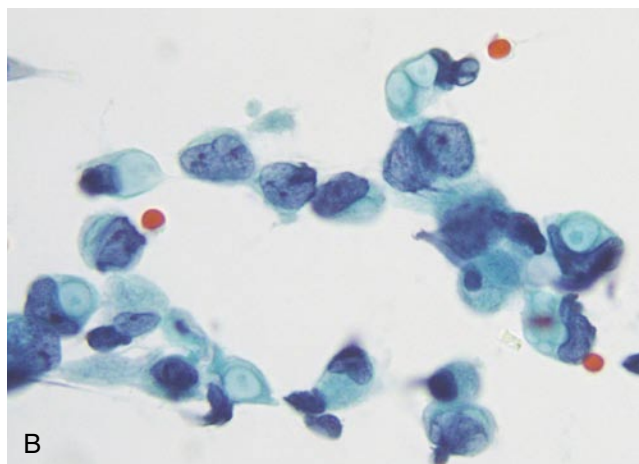


Figure 8.27 Ductal carcinoma. The tumor cells are buried in a background of marked acute inflammation. In samples with abundant acute inflammation, a careful search must be conducted to exclude malignant cells (Papanicolaou stain).



A



B

Figure 8.28 Lobular carcinoma. A, A loose single-file arrangement is apparent. B, Large solitary intracytoplasmic vacuoles are present, giving a signet ring cell appearance (Papanicolaou stain).

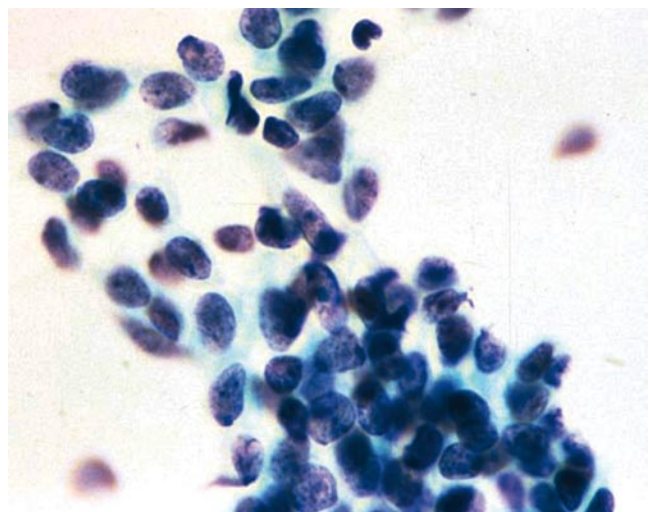


Figure 8.29 Lobular carcinoma in-situ (LCIS). The cells are present in loosely cohesive sheets (Papanicolaou stain).

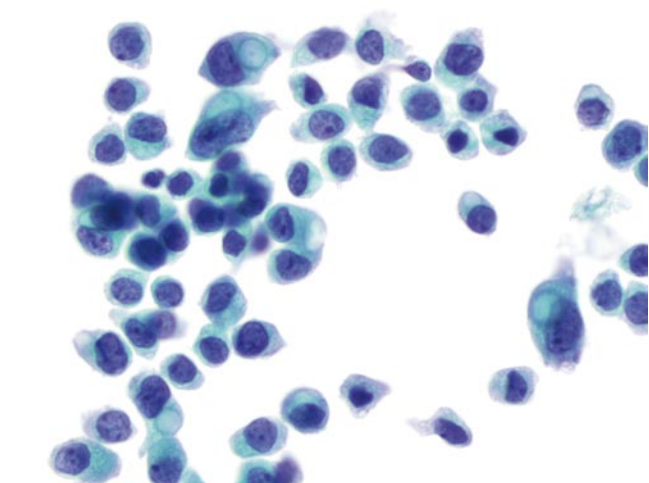


Figure 8.30 Lobular carcinoma in-situ (LCIS). In contrast to the previous case (see Fig. 8.29), the cells are dispersed singly and signet cell forms are noted. Such a case is likely to be over-diagnosed as a lobular carcinoma (Papanicolaou stain).

Medullary Carcinoma

Medullary carcinoma (MC) accounts for 1% to 7% of breast tumors.¹⁷³ It is a well-circumscribed mass composed of large, poorly differentiated cells with scant stroma and a marked lymphoid infiltrate. Hemorrhage and necrosis occur in some cases; as a result MCs can be cystic.^{72,204} Because they are well-circumscribed, they can be mistaken clinically for a cyst or FA.

CYTOMORPHOLOGY OF MEDULLARY CARCINOMA:

- hypercellular smears
- numerous isolated cells and loose clusters
- markedly enlarged, vesicular nucleus

- prominent and often irregular macronucleolus
- numerous mitoses
- granular scarce to abundant cytoplasm
- many lymphocytes and some plasma cells

DIFFERENTIAL DIAGNOSIS OF MEDULLARY CARCINOMA:

- chronic mastitis
- intramammary lymph node
- lymphoma
- IDC

Chronic mastitis and an intramammary lymph node lack the abundant large, poorly differentiated tumor cells of MC.²⁰⁵ The tumor cells of MC are larger and more variable than those of large cell lymphoma, and they often show some clustering, an uncommon feature in most lymphomas. In ambiguous cases, immunocytochemistry for leukocyte common antigen (LCA), keratin, and epithelial membrane antigen (EMA) are helpful. The difference between MC and poorly differentiated ductal carcinoma is virtually impossible to discern cytologically.^{205,206} The distinction is based on histologic criteria such as circumscription of the tumor; FNA can only suggest the subclassification of a malignant tumor as MC. The distinction is important because patients with MC have a significantly better prognosis than those with IDC.

Mucinous (Colloid) Carcinoma

This distinct type of invasive carcinoma is composed entirely of aggregates of uniform cells floating in abundant extracellular mucus. Mucinous carcinomas comprise about 2% of invasive breast cancers, but focal mucinous differentiation can be seen in an additional 2% of breast carcinomas.¹⁷³ These slow-growing tumors confer a better prognosis than that of IDC. It has been suggested that FNA is significantly less sensitive than core needle biopsy in this setting.²⁰⁷ These tumors can be suggested²⁰⁸ with terminology such as “carcinoma with prominent mucinous features,” but a distinction between a mucinous carcinoma and an IDC with focal mucinous change is not possible by FNA.²⁰⁹

CYTOMORPHOLOGY OF MUCINOUS CARCINOMA:

- tightly cohesive three-dimensional balls of cells (Fig. 8.31)
- mucinous background staining red-violet with a Romanowsky-type stain and green-purple with a Papanicolaou stain
- branching capillary structures (Fig. 8.32)

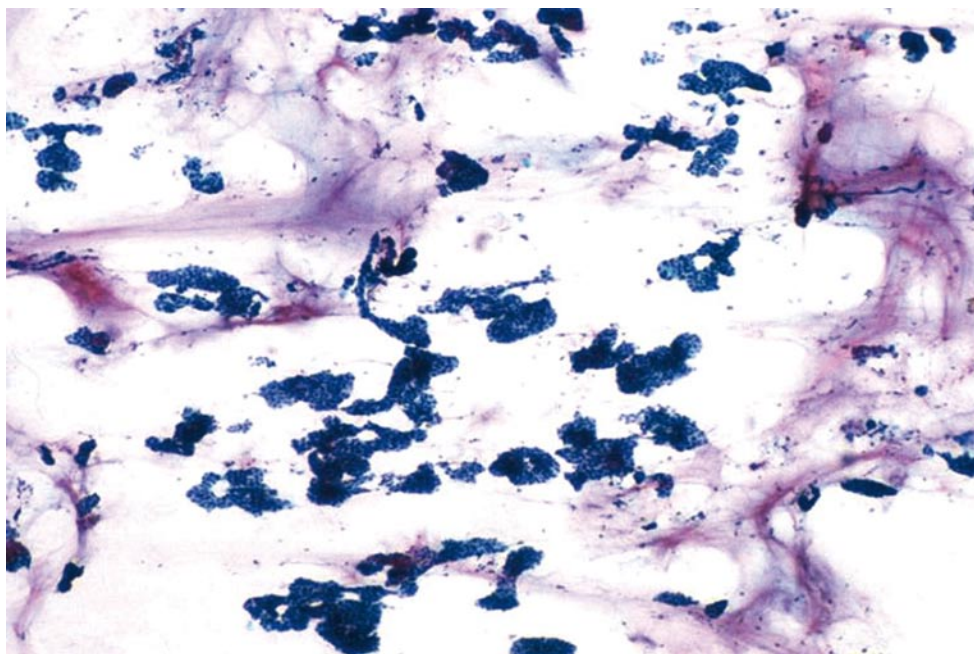


Figure 8.31 Mucinous carcinoma. At low power, numerous tightly cohesive clusters dispersed in a mucinous background are noted (Papanicolaou stain).

- uniform, unremarkable nucleus (Fig. 8.33)
- small vacuoles in the cytoplasm
- plasmacytoid cells with an eccentric nucleus^{210, 211}
- rarely psammoma bodies^{210, 211}

DIFFERENTIAL DIAGNOSIS OF MUCINOUS CARCINOMA:

- mucocele
- FA
- lobular carcinoma
- invasive carcinoma with focal mucinous features

A mucocele is a benign mucin-filled cyst that often ruptures. Mucoceles lack the abundant three-dimensional balls of neoplastic cells that are typical of mucinous carcinoma.^{212,213} Although an FA can also have a myxoid background, it is more cellular, and the cells are arranged in large groups or in antler-like configurations rather than balls.²¹⁴ In addition, FAs may have many single stromal or bipolar cells or stripped nuclei and myxoid stromal fragments. Lobular carcinoma is often composed of vacuolated cells, but these are commonly arranged as isolated cells and not as balls, and a mucinous background is absent. Because a definite distinction between a mucinous carcinoma and a mixed mucinous and typical ductal tumor is not possible, terminology such as “carcinoma with prominent mucinous features” rather than an outright diagnosis of “mucinous carcinoma” is preferred.²⁰⁹

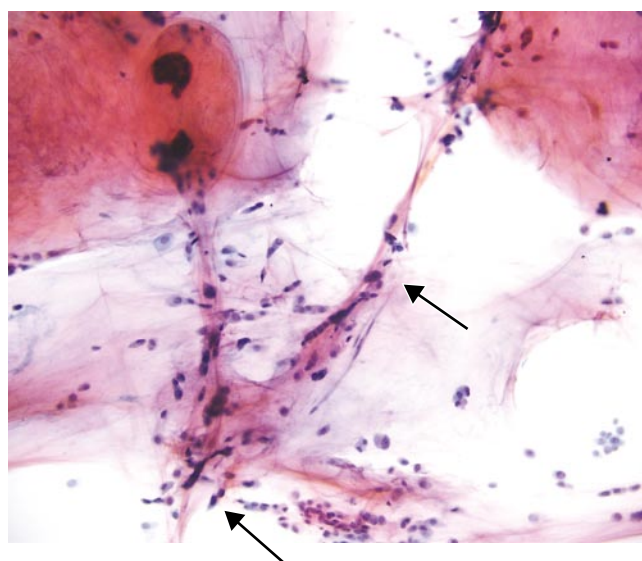


Figure 8.32 Mucinous carcinoma. Branching capillary structures in a mucinous background should suggest the diagnosis. Single cells may be seen in addition to cellular balls (Papanicolaou stain).

Tubular Carcinoma

Tubular carcinoma (TC), a well-differentiated tumor comprising less than 2% of breast carcinomas, is composed of well-defined tubules lined by a single layer of neoplastic cells and surrounded by a dense fibrous stroma. Because some IDCs can have small foci of TC, the specific diagnosis of TC is reserved for cases in which well-defined tubules constitute more than 75%

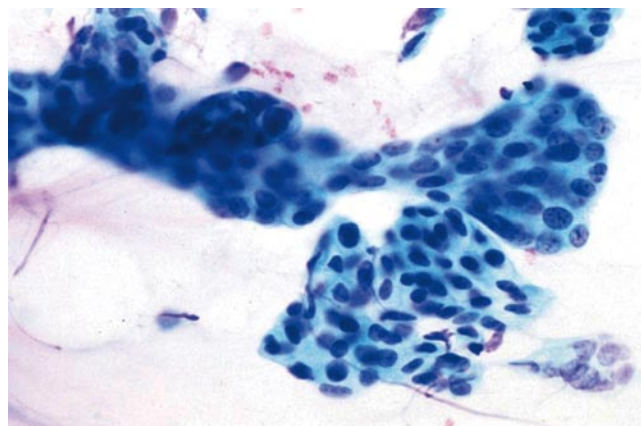


Figure 8.33 Mucinous carcinoma. At higher power, the low-grade round, regular nuclei are noted (Papanicolaou stain).

of the total tumor. Defined in this way, the prognosis for patients with TC is more favorable than for those with IDC. The sensitivity for the diagnosis of TC is lower for FNA than for core biopsy (50% versus 91%, respectively), and fewer cases receive an outright diagnosis of malignancy (42% versus 73%, respectively).^{215,216}

CYTOMORPHOLOGY OF TUBULAR CARCINOMA:

- hypocellular smear because of the dense fibrosis
- predominantly cohesive, often angular clusters (sometimes described as comma shaped or cornucopia shaped, Figs. 8.34, 8.35)
- peripheral perpendicular cells around tubular clusters²¹⁷
- some dyshesion
- uniform, medium-sized tumor cells with a round, uniform nucleolus?
- finely granular chromatin
- small nucleolus
- occasional cells have a large cytoplasmic vacuole

DIFFERENTIAL DIAGNOSIS OF TUBULAR CARCINOMA:

- FA
- proliferative ductal lesions
- IDC
- invasive lobular carcinoma

TC is well known to yield false-negative FNA results. Tumor cells tend to be arranged in smaller groups than those of FA or ductal proliferative lesions. The presence of angular epithelial groups, isolated epithelial cells, and nuclear atypia, warrants consideration of the diagnosis of TC.²¹⁶⁻²¹⁸ The cytologic features of TC overlap significantly with well-differentiated ductal carcinoma and lobular carcinoma, both of which can have a minor

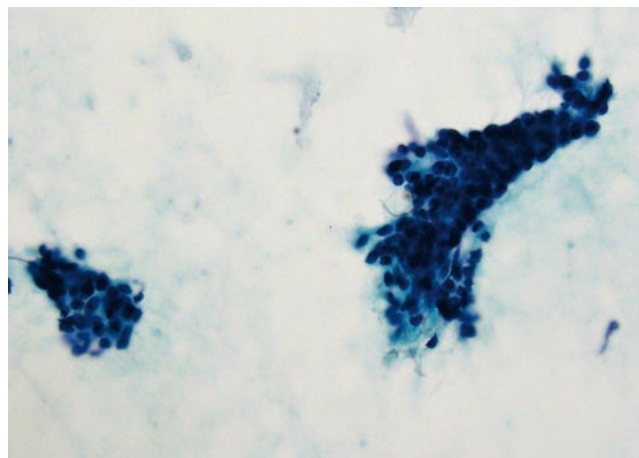


Figure 8.34 Tubular carcinoma. Cells in tightly cohesive clusters often have rigid borders. Nuclear atypia is minimal, and single cells are usually not seen (Papanicolaou stain).

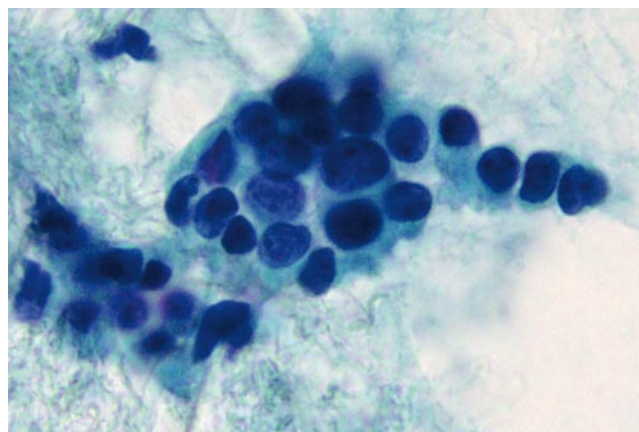


Figure 8.35 Tubular carcinoma. Clusters of cells typically come to a sharp point (comma or cornucopia formations). By contrast, fibroadenomas (FAs) tend to have more rounded and less rigid outlines (Papanicolaou stain).

component of TC. A definite diagnosis depends on a surgical biopsy.

Metaplastic Carcinomas

Metaplastic carcinomas, which represent less than 1% of breast carcinomas, are a heterogeneous group of carcinomas with mesenchymal or squamous differentiation. In some cases, evidence of ductal differentiation is entirely lacking and the tumor is composed exclusively of squamous or sarcomatoid elements. Some tumors can be cystic.

CYTOMORPHOLOGY OF METAPLASTIC CARCINOMAS:

- moderate to marked cytologic atypia
- clusters of and isolated tumor cells

- large, pleomorphic, and spindle-shaped cells
- malignant squamous or glandular cells
- benign multinucleated giant cells
- rarely, malignant cartilage or bone²¹⁹
- background of amorphous debris and inflammatory cells

DIFFERENTIAL DIAGNOSIS OF METAPLASTIC CARCINOMAS:

- FCC
- subareolar abscess
- benign squamous metaplasia following lumpectomy and irradiation
- PT
- sarcoma
- spindle cell DCIS
- angiosarcoma

Metaplastic carcinoma should be considered when there are high-grade malignant features with mesenchymal or combined epithelial and mesenchymal elements, and especially with two or more elements.^{184,220-224} Cystic tumors can yield few if any malignant cells, and some multinucleated giant cells resemble the foam cells seen in cysts of FCC. A squamous component with inflammation may suggest a subareolar abscess, but a peripheral location favors the diagnosis of metaplastic carcinoma. Squamous metaplasia following lumpectomy and irradiation is more problematic in that significant atypia may be encountered.²²⁵ In a PT, the mesenchymal element is usually arranged in stromal fragments rather than as

isolated cells, and abundant benign epithelium is present. A primary breast sarcoma can be cytologically indistinguishable from a sarcomatoid metaplastic carcinoma.²²⁶ Because metaplastic carcinoma is an epithelial neoplasm, immunocytochemistry can be helpful, because it is immunoreactive for cytokeratin and EMA. Spindle cell DCIS is a rare entity that may be indistinguishable from metaplastic carcinoma.²²⁷ Angiosarcomas may present as a secondary lesion after breast carcinoma. The cells may occasionally appear spindle-shaped and epithelial. Immunohistochemical stains for the endothelial markers CD31 and CD34 are a useful adjunct.²²⁸

UNCOMMON BREAST TUMORS

Apocrine Carcinoma

As its name indicates, apocrine carcinoma is composed predominantly of apocrine cells - that is, cells which resemble the apocrine metaplasia often seen in FCC. This type of cancer constitutes about 4% of breast cancers.¹⁷³ It is clinically indistinguishable from IDC.

CYTOMORPHOLOGY OF APOCRINE CARCINOMA:

- generally hypercellular specimens (Fig. 8.36)
- clusters and sheets of and isolated cells
- abundant granular cytoplasm with indistinct cell borders
- enlarged nucleus with irregular contours
- prominent large nucleolus (Fig. 8.37)
- necrotic debris

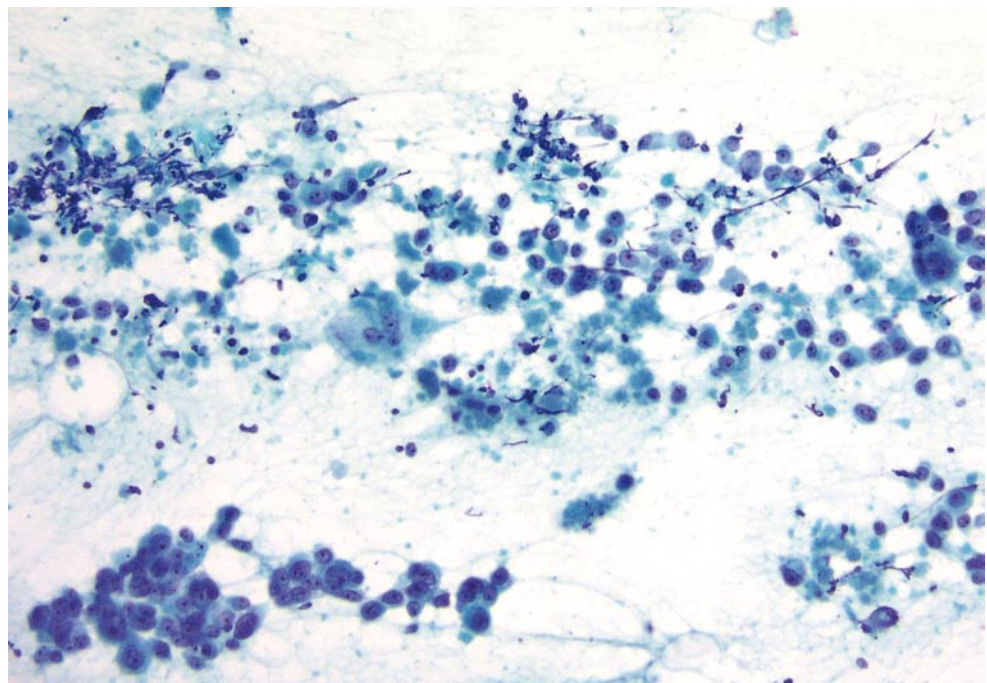


Figure 8.36 Apocrine carcinoma. A variant of ductal carcinoma, apocrine carcinoma is also typified by hypercellularity, nuclear atypia, and many single cells (Papanicolaou stain).

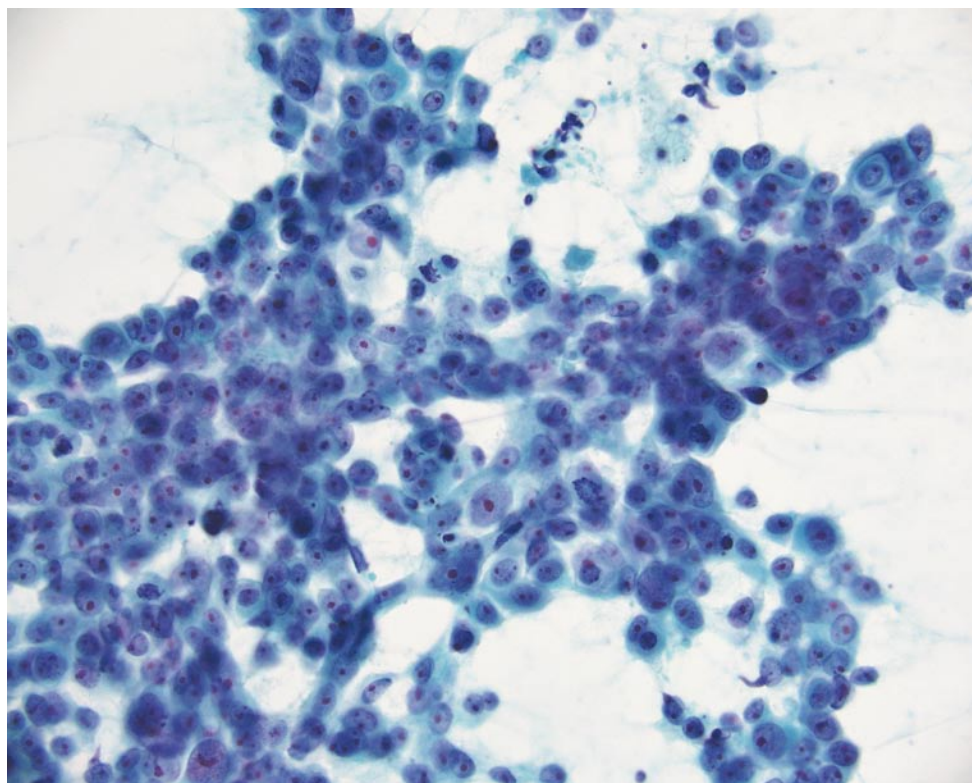


Figure 8.37 Apocrine carcinoma. Note the abundant granular cytoplasm, pronounced nuclear atypicity and prominent nucleoli, which are round and centrally located (Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF APOCRINE CARCINOMA:

- apocrine metaplasia
- apocrine adenosis

Although apocrine metaplasia can have significant variation in nuclear size, marked nuclear atypia is generally not observed.²²⁹ The presence of protruding nuclei (comet-shaped) is also useful because these are seen in carcinoma, whereas nuclei are centrally located in apocrine metaplasia. Apocrine carcinoma is almost invariably a solid mass clinically and radiologically, and necrosis is not present in fibrocystic change. Apocrine adenosis, a distinct histologic entity, may overlap with apocrine carcinoma but usually has many naked nuclei and less nuclear hyperchromasia.¹³⁰

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma represents about 0.1% of breast cancers and is morphologically and cytologically identical to its namesakes in the salivary glands and other sites.²³⁰⁻²³⁶ An immunostain for collagen IV stains the background material of adenoid cystic carcinoma.²³⁷ Patients with adenoid cystic carcinoma of the breast,

unlike those with its salivary gland counterpart, have an excellent prognosis.

CYTOMORPHOLOGY OF ADENOID CYSTIC CARCINOMA:

- hypercellular smears
- nests of cohesive small cells (Fig. 8.38)
- uniform round or oval nucleus and scant cytoplasm
- hyperchromatic nucleus with coarsely granular chromatin and small nucleolus
- round globules within the nests that stain bright red or purple with a Romanowsky stain and pale green with Papanicolaou stain (Fig. 8.39)
- p63 stains tumor cells in 85% of cases of adenoid cystic carcinoma (Fig. 8.40)²³⁸

DIFFERENTIAL DIAGNOSIS OF ADENOID CYSTIC CARCINOMA:

- collagenous spherulosis
- adenomyoepithelioma

Similar globules are seen in collagenous spherulosis associated with benign ductal hyperplasia.^{235,239,240} Adenomyoepithelioma is another rare tumor composed of myoepithelial cells that must be distinguished from

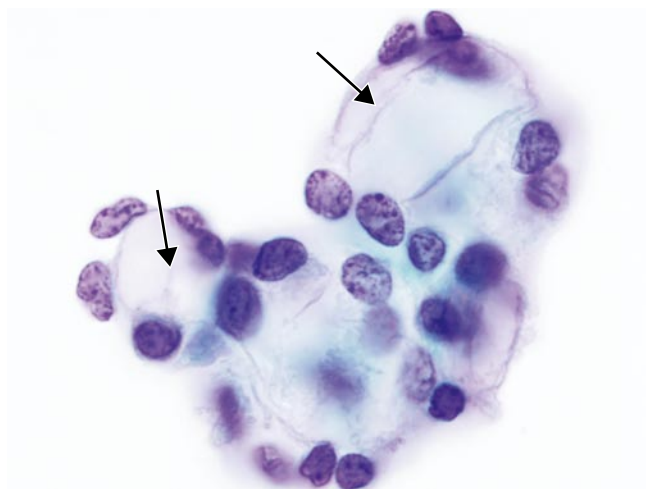


Figure 8.38 Adenoid cystic carcinoma. Tightly cohesive clusters of cells with round, uniform nuclei are noted. Globules are subtle and appear somewhat refractile in this case (ThinPrep Papanicolaou stain).

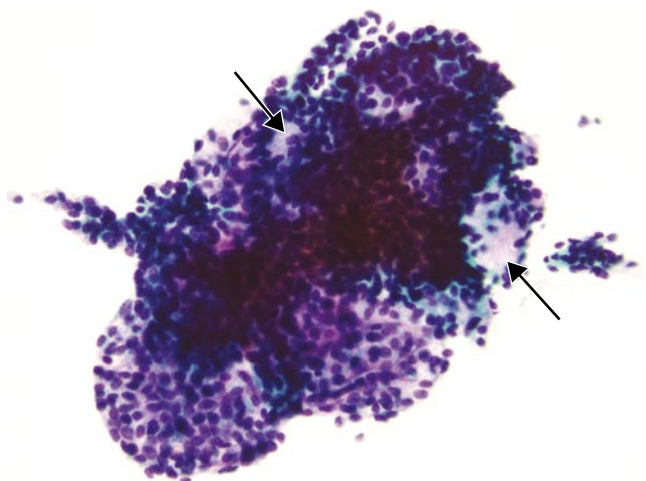


Figure 8.39 Adenoid cystic carcinoma. Hyaline globules are somewhat more prominent within aggregates of cells. They also stain pale green with Papanicolaou stain (ThinPrep).

adenoid cystic carcinoma. The cells of adenomyoepithelioma are arranged in tightly cohesive clusters with scant stromal material, but lack the typical hyaline globules of adenoid cystic carcinoma.^{241,242}

Non-Hodgkin Lymphoma

Non-Hodgkin lymphoma can involve the breast either as a primary neoplasm or secondary to systemic disease that also involves lymph nodes. The majority have a B-cell phenotype, and the most subtypes are diffuse large B-cell lymphomas (DLBL), follicular center cell lymphomas, and lymphomas of mucosa-associated lymphoid tissue (MALT).²⁴³ The cytologic features are identical to

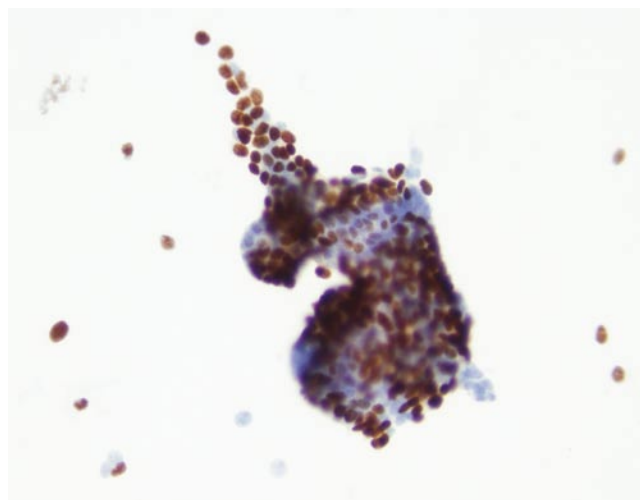


Figure 8.40 Adenoid cystic carcinoma. Nuclear immunoreactivity for p63 is a useful adjunct test when considering this lesion. (ThinPrep, immunohistochemical stain).

those of lymphomas that arise in lymph nodes.²⁰⁵ The use of flow cytometry can improve subclassification significantly.²⁴⁴ Rarely plasmacytoma or myeloma can also involve the breast with typical findings.^{245,246}

CYTOMORPHOLOGY OF NON-HODGKIN LYMPHOMA:

- single cell pattern with lymphoglandular bodies (best seen with Romanowsky-type stains)
- monomorphic, atypical cells, often with irregular nuclear contours and a prominent nucleolus

DIFFERENTIAL DIAGNOSIS OF NON-HODGKIN LYMPHOMA:

- chronic mastitis
- intramammary lymph node
- LA

Sarcoma

Primary sarcomas of the breast are rare and include liposarcoma, osteosarcoma, leiomyosarcoma, rhabdomyosarcoma, hemangiopericytoma, and angiosarcoma. The most common is angiosarcoma.²⁴³ Features are similar to the findings on soft tissue aspiration.^{228,247-251}

CYTOMORPHOLOGY OF ANGIOSARCOMA:

- similar to soft tissue sarcoma
- cells singly and in clusters
- hyperchromatic nuclei
- coarsely granular chromatin pattern
- nucleolus often prominent

DIFFERENTIAL DIAGNOSIS OF ANGIOSARCOMA:

- PT
- metaplastic carcinoma

METASTATIC TUMORS

Nonmammary tumors metastasize to the breast parenchyma, and in some cases the primary tumor is occult. Common suspects in such clinically occult cases are lung

cancer, melanoma, renal cell carcinoma, adenocarcinoma of the stomach, and intestinal carcinoid tumors.⁶⁹

CYTOMORPHOLOGY OF METASTATIC TUMORS:

- Consider whenever the cytologic findings are not typical for breast carcinoma (Figs. 8.41, 8.42)

If the site of the primary tumor is known, comparison of the cytology with available prior morphology is often helpful for distinguishing a primary breast cancer from a metastasis.

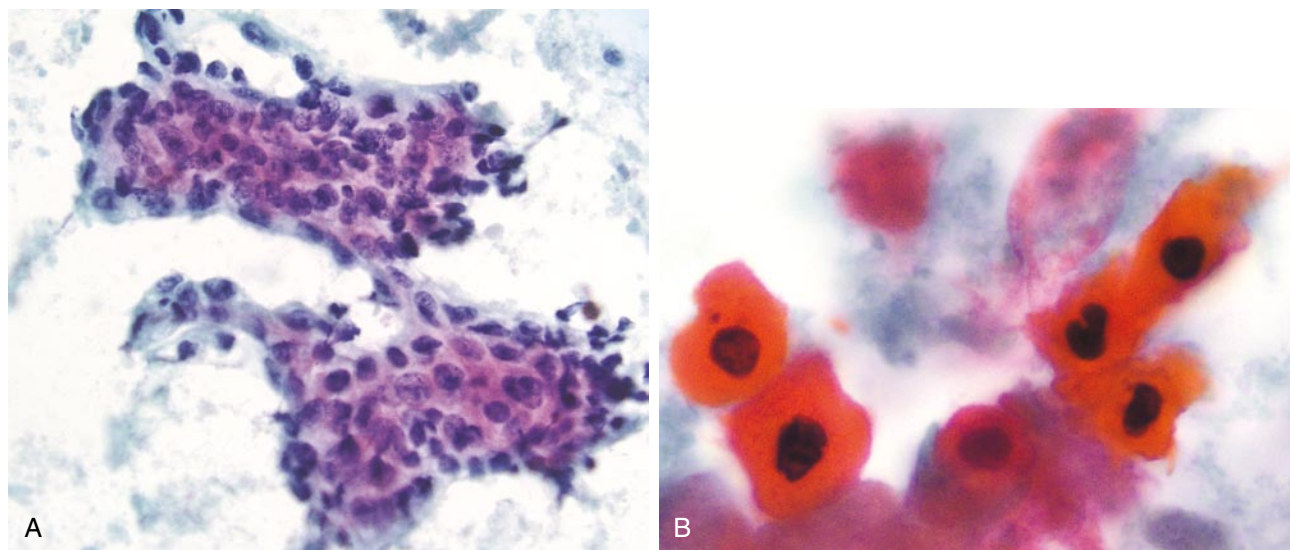


Figure 8.41 Metastatic squamous cell carcinoma to the breast. Although the loosely cohesive aggregate of markedly atypical cells in A, resembles a poorly differentiated breast carcinoma, the malignant keratinized squamous cells in B, raise the suspicion of an unusual tumor. This was a metastasis from a squamous carcinoma of the vulva (Papanicolaou stain).

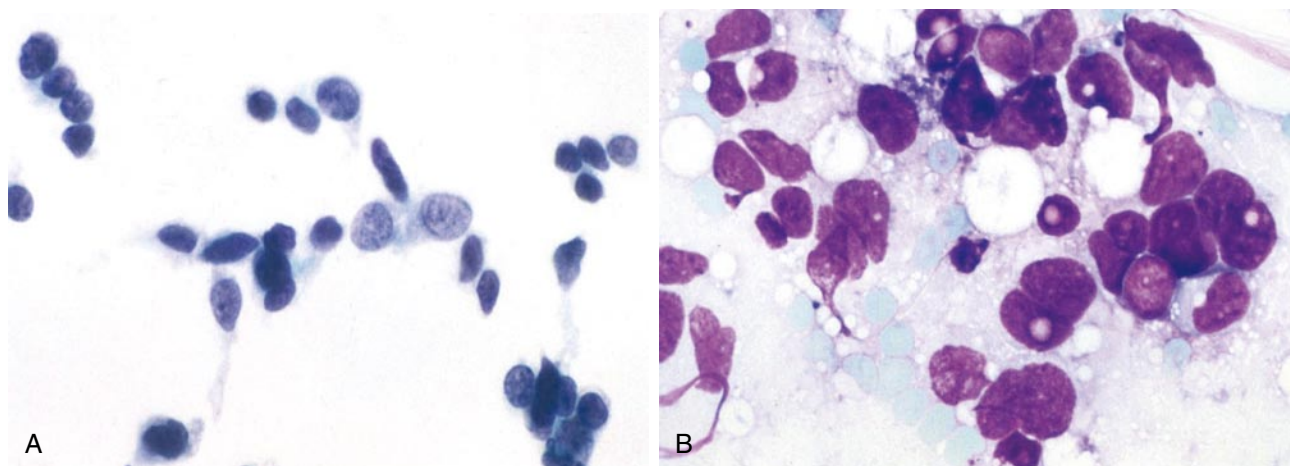


Figure 8.42 Metastatic small cell carcinoma to the breast. The cells from a metastatic small cell carcinoma to the breast might be mistaken for lobular carcinoma, but there is greater nuclear atypia and pleomorphism, less cytoplasm, and pronounced nuclear molding (A, Papanicolaou stain, B, Diff-Quik stain).

REFERENCES

- Brenner RJ, Bassett LW, Fajardo LL, et al.: Stereotactic core-needle breast biopsy: A multi-institutional prospective trial. *Radiology* 2001;218(3):866-872.
- Tabbara SO, Frost AR, Stoler MH, et al.: Changing trends in breast fine-needle aspiration: Results of the Papanicolaou Society of Cytopathology Survey. *Diagn Cytopathol* 2000;22(2):126-130.
- Fajardo LL, Pisano ED, Caudry DJ, et al.: Stereotactic and sonographic large-core biopsy of nonpalpable breast lesions: Results of the Radiologic Diagnostic Oncology Group V study. *Acad Radiol* 2004;11(3):293-308.
- Masood S: Core needle biopsy versus fine needle aspiration biopsy: Are there similar sampling and diagnostic issues? *Clin Lab Med* 2005;25(4):679-688, vi.
- Symmans WF, Cangiarella JF, Gottlieb S, et al.: What is the role of cytopathologists in stereotaxic needle biopsy diagnosis of nonpalpable mammographic abnormalities? *Diagn Cytopathol* 2001;24(4):260-270.
- Sneige N, Tulbah A: Accuracy of cytologic diagnoses made from touch imprints of image-guided needle biopsy specimens of nonpalpable breast abnormalities. *Diagn Cytopathol* 2000;23(1):29-34.
- Albert US, Duda V, Hadji P, et al.: Imprint cytology of core needle biopsy specimens of breast lesions. A rapid approach to detecting malignancies, with comparison of cytologic and histopathologic analyses of 173 cases. *Acta Cytol* 2000;44(1):57-62.
- March DE, Walker MT, Bur M, et al.: Touch-preparation cytologic examination of breast core biopsy specimens: accuracy in predicting benign or malignant core histologic results. *Acad Radiol* 1999;6(6):333-338.
- Jacobs TW, Silverman JF, Schroeder B, et al.: Accuracy of touch imprint cytology of image-directed breast core needle biopsies. *Acta Cytol* 1999;43(2):169-174.
- Lankford KV, Kluskens L, Dowlathshahi K, et al.: Utilization of core wash material in the diagnosis of breast lesions by stereotactic needle biopsy. *Cancer* 1998;84(2):98-100.
- Farshid G, Pieterse S: Core imprint cytology of screen-detected breast lesions is predictive of the histologic results. *Cancer* 2006;108(3):150-156.
- Leifland K, Lagerstedt U, Svane G: Comparison of stereotactic fine needle aspiration cytology and core needle biopsy in 522 non-palpable breast lesions. *Acta Radiol* 2003;44(4):387-391.
- Pisano ED, Fajardo LL, Tsimikas J, et al.: Rate of insufficient samples for fine-needle aspiration for nonpalpable breast lesions in a multicenter clinical trial: The Radiologic Diagnostic Oncology Group 5 Study. The RDOG5 investigators. *Cancer* 1998;82(4):679-688.
- Westenend PJ, Sever AR, Beekman-De Volder HJ, Liem SJ: A comparison of aspiration cytology and core needle biopsy in the evaluation of breast lesions. *Cancer* 2001;93(2):146-150.
- Rubin E, Mennemeyer ST, Desmond RA, et al.: Reducing the cost of diagnosis of breast carcinoma: Impact of ultrasound and imaging-guided biopsies on a clinical breast practice. *Cancer* 2001;91(2):324-332.
- Hatada T, Ishii H, Ichii S, et al.: Diagnostic value of ultrasound-guided fine-needle aspiration biopsy, core-needle biopsy, and evaluation of combined use in the diagnosis of breast lesions. *J Am Coll Surg* 2000;190(3):299-303.
- Symmans WF, Weg N, Gross J, et al.: A prospective comparison of stereotaxic fine-needle aspiration versus stereotaxic core needle biopsy for the diagnosis of mammographic abnormalities. *Cancer* 1999;85(5):1119-1132.
- Lifrange E, Kridelka F, Colin C: Stereotaxic needle-core biopsy and fine-needle aspiration biopsy in the diagnosis of nonpalpable breast lesions: controversies and future prospects. *Eur J Radiol* 1997;24(1):39-47.
- Bulgaresi P, Cariaggi P, Ciatto S, Houssami N: Positive predictive value of breast fine needle aspiration cytology (FNAC) in combination with clinical and imaging findings: A series of 2334 subjects with abnormal cytology. *Breast Cancer Res Treat* 2006;97(3):319-321.
- Lieske B, Ravichandran D, Wright D: Role of fine-needle aspiration cytology and core biopsy in the preoperative diagnosis of screen-detected breast carcinoma. *Br J Cancer* 2006;95(1):62-66.
- Logan-Young W, Dawson AE, Wilbur DC, et al.: The cost-effectiveness of fine-needle aspiration cytology and 14-gauge core needle biopsy compared with open surgical biopsy in the diagnosis of breast carcinoma. *Cancer* 1998;82(10):1867-1873.
- Hatmaker AR, Donahue RM, Tarpley JL, Pearson AS: Cost-effective use of breast biopsy techniques in a Veterans health care system. *Am J Surg* 2006;192(5):e37-e41.
- Adler DD, Light RJ, Granstrom P, et al.: Follow-up of benign results of stereotactic core breast biopsy. *Acad Radiol* 2000;7(4):248-253.
- Burak WE Jr, Owens KE, Tighe MB, et al.: Vacuum-assisted stereotactic breast biopsy: histologic underestimation of malignant lesions. *Arch Surg* 2000;135(6):700-703.
- Houssami N, Ciatto S, Ellis I, Ambrogetti D: Underestimation of malignancy of breast core-needle biopsy: Concepts and precise overall and category-specific estimates. *Cancer* 2007;109(3):487-495.
- Ciatto S, Houssami N, Ambrogetti D, et al.: Accuracy and underestimation of malignancy of breast core needle biopsy: The Florence experience of over 4000 consecutive biopsies. *Breast Cancer Res Treat* 2007;101(3):291-297.
- Denley H, Pinder SE, Elston CW, et al.: Preoperative assessment of prognostic factors in breast cancer. *J Clin Pathol* 2001;54(1):20-24.
- Clarke D, Sudhakaran N, Gateley CA: Replace fine needle aspiration cytology with automated core biopsy in the triple assessment of breast cancer. *Ann R Coll Surg Engl* 2001;83(2):110-112.
- Ibrahim AE, Bateman AC, Theaker JM, et al.: The role and histological classification of needle core biopsy in comparison with fine needle aspiration cytology in the preoperative assessment of impalpable breast lesions. *J Clin Pathol* 2001;54(2):121-125.
- Britton PD, Flower CD, Freeman AH, et al.: Changing to core biopsy in an NHS breast screening unit. *Clin Radiol* 1997;52(10):764-767.
- Duijm LE, Groenewoud JH, Roumen RM, et al.: A decade of breast cancer screening in The Netherlands: Trends in the preoperative diagnosis of breast cancer. *Breast Cancer Res Treat* 2007;106(1):113-119.
- Xie HB, Salhadar A, Haara A, et al.: How stereotactic core-needle biopsy affected breast fine-needle aspiration utilization: An 11-year institutional review. *Diagn Cytopathol* 2004;31(2):106-110.
- Florio MG, Manganaro T, Pollicino A, et al.: Surgical approach to nipple discharge: A ten-year experience. *J Surg Oncol* 1999;71(4):235-238.
- Jayaram G, Elsayed EM, Yaccob RB: Papillary breast lesions diagnosed on cytology Profile of 65 cases. *Acta Cytol* 2007;51(1):3-8.
- Ciatto S, Bravetti P, Cariaggi P: Significance of nipple discharge clinical patterns in the selection of cases for cytologic examination. *Acta Cytol* 1986;30(1):17-20.
- Knight DC, Lowell DM, Heimann A, Dunn E: Aspiration of the breast and nipple discharge cytology. *Surg Gynecol Obstet* 1986;163(5):415-420.

37. Takeda T, Matsui A, Sato Y, et al.: Nipple discharge cytology in mass screening for breast cancer. *Acta Cytol* 1990;34(2):161-164.
38. Markopoulos C, Mantas D, Kouskos E, et al.: Surgical management of nipple discharge. *Eur J Gynaecol Oncol* 2006;27(3):275-278.
39. Sauter ER, Wagner-Mann C, Ehya H, Klein-Szanto A: Biologic markers of breast cancer in nipple aspirate fluid and nipple discharge are associated with clinical findings. *Cancer Detect Prev* 2007;31(1):50-58.
40. Sauter ER, Ross E, Daly M, et al.: Nipple aspirate fluid: A promising non-invasive method to identify cellular markers of breast cancer risk. *Br J Cancer* 1997;76(4):494-501.
41. Wrensch MR, Petrakis NL, Miike R, et al.: breast cancer risk in women with abnormal cytology in nipple aspirates of breast fluid. *J Natl Cancer Inst* 2001;93(23):1791-1798.
42. Sauter ER, Ehya H, Babb J, et al.: Biological markers of risk in nipple aspirate fluid are associated with residual cancer and tumour size. *Br J Cancer* 1999;81(7):1222-1227.
43. Hou M, Tsai K, Lin H, et al.: A simple intraductal aspiration method for cytodiagnosis in nipple discharge. *Acta Cytol* 2000;44(6):1029-1034.
44. Fabian CJ, Kimler BF: Breast cancer risk prediction: Should nipple aspiration fluid cytology be incorporated into clinical practice? *J Natl Cancer Inst* 2001;93(23):1762-1763.
45. Pereira B, Mokbel K: Mammary ductoscopy: Past, present, and future. *Int J Clin Oncol* 2005;10(2):112-116.
46. Shen KW, Wu J, Lu JS, et al.: Fiberoptic ductoscopy for patients with nipple discharge. *Cancer* 2000;89(7):1512-1519.
47. Francescatti DS, Kluskens L, Shah L: Ductal lavage in the high-risk patient. *Am J Surg* 2005;189(3):340-341.
48. Masood S, Khalbuss WE: Nipple fluid cytology. *Clin Lab Med* 2005;25(4):787-794, vii-viii.
49. Vogel VG: Atypia in the assessment of breast cancer risk: implications for management. *Diagn Cytopathol* 2004;30(3):151-157.
50. Ljung BM, Chew KL, Moore DH 2nd, King EB: Cytology of ductal lavage fluid of the breast. *Diagn Cytopathol* 2004;30(3):143-150.
51. O'Shaughnessy JA, Ljung BM, Dooley WC, et al.: Ductal lavage and the clinical management of women at high risk for breast carcinoma. *Cancer* 2002;94:292-298.
52. Kurian AW, Mills MA, Jaffee M, et al.: Ductal lavage of fluid-yielding and non-fluid-yielding ducts in BRCA1 and BRCA2 mutation carriers and other women at high inherited breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2005;14(5):1082-1089.
53. Li J, Zhao J, Yu X, et al.: Identification of biomarkers for breast cancer in nipple aspiration and ductal lavage fluid. *Clin Cancer Res* 2005;11(23):8312-8320.
54. Fabian CJ: Is there a future for ductal lavage? *Clin Cancer Res* 2007;13(16):4655-4666.
55. Badve S: Ductal lavage and its histopathologic basis: a cautionary tale. *Diagn Cytopathol* 2004;30(3):166-171.
56. Arun B, Valero V, Logan C, et al.: Comparison of ductal lavage and random periareolar fine needle aspiration as tissue acquisition methods in early breast cancer prevention trials. *Clin Cancer Res* 2007;13(16):4943-4948.
57. Zalles CM, Kimler BF, Simonsen M, Clark JL, Metheny T, Fabian CJ: Comparison of cytomorphology in specimens obtained by random periareolar fine needle aspiration and ductal lavage from women at high risk for development of breast cancer. *Breast Cancer Res Treat* 2006;97(2):191-197.
58. Visvanathan K, Santor D, Ali SZ, et al.: The reliability of nipple aspirate and ductal lavage in women at increased risk for breast cancer—A potential tool for breast cancer risk assessment and biomarker evaluation. *Cancer Epidemiol Biomarkers Prev* 2007;16(5):950-955.
59. Sardanelli F, Imperiale A, Zandrino F, et al.: Breast intraductal masses: US-guided fine-needle aspiration after galactography. *Radiology* 1997;204(1):143-148.
60. Ciatto S, Brancato B, Rizzo G, et al.: Accuracy of fine needle aspiration cytology (FNAC) of axillary lymph nodes as a triage test in breast cancer staging. *Breast Cancer Res Treat* 2007;103(1):85-91.
61. Somasundar P, Gass J, Steinhoff M, et al.: Role of ultrasound-guided axillary fine-needle aspiration in the management of invasive breast cancer. *Am J Surg* 2006;192(4):458-461.
62. Popli MB, Sahoo M, Mehrotra N, et al.: Preoperative ultrasound-guided fine-needle aspiration cytology for axillary staging in breast carcinoma. *Australas Radiol* 2006;50(2):122-126.
63. Duchesne N, Jaffey J, Florack P, Duchesne S: Redefining ultrasound appearance criteria of positive axillary lymph nodes. *Can Assoc Radiol J* 2005;56(5):289-296.
64. Brancato B, Zappa M, Bricolo D, et al.: Role of ultrasound-guided fine needle cytology of axillary lymph nodes in breast carcinoma staging. *Radiol Med (Torino)* 2004;108(4):345-355.
65. Kuenen-Boumeester V, Menke-Pluymers M, de Kanter AY, et al.: Ultrasound-guided fine needle aspiration cytology of axillary lymph nodes in breast cancer patients. A preoperative staging procedure. *Eur J Cancer* 2003;39(2):170-174.
66. Bedard YC, Pollett AF: Breast fine-needle aspiration A comparison of ThinPrep and conventional smears. *Am J Clin Pathol* 1999;111(4):523-527.
67. Florentine BD, Wu NC, Waliany S, et al.: Fine needle aspiration (FNA) biopsy of palpable breast masses: comparison of conventional smears with the Cyto-Tek MonoPrep system. *Cancer* 1999;87(5):278-285.
68. Pinto RG, Couto F, Mandreker S: Infarction after fine needle aspiration. A report of four cases. *Acta Cytol* 1996;40(4):739-741.
69. Rosen PP, Oberman HA (eds): *Tumors of the Mammary Gland*. Washington, DC, Armed Forces Institute of Pathology, 1993.
70. Venta LA, Kim JB, Pelloski CE, Morrow M: Management of complex breast cysts. *AJR Am J Roentgenol* 1999;173(5):1331-1336.
71. Smith DN, Kaelin CM, Korbin CD, et al.: Impalpable breast cysts: Utility of cytologic examination of fluid obtained with radiologically guided aspiration. *Radiology* 1997;204(1):149-151.
72. Howell LP, Kline TS: Medullary carcinoma of the breast. An unusual cytologic finding in cyst fluid aspirates. *Cancer* 1990;65(2):277-282.
73. Berg WA, Campassi CI, Ioffe OB: Cystic lesions of the breast: sonographic-pathologic correlation. *Radiology* 2003;227(1):183-191.
74. Collaco LM, de Lima RS, Werner B, Torres LF: Value of fine needle aspiration in the diagnosis of breast lesions. *Acta Cytol* 1999;43(4):587-592.
75. O'Neil S, Castelli M, Gattuso P, et al.: Fine-needle aspiration of 697 palpable breast lesions with histopathologic correlation. *Surgery* 1997;122(4):824-828.
76. Giard RW, Hermans J: The value of aspiration cytologic examination of the breast. A statistical review of the medical literature. *Cancer* 1992;69(8):2104-2110.
77. Arisio R, Cuccorese C, Accinelli G, et al.: Role of fine-needle aspiration biopsy in breast lesions: Analysis of a series of 4,110 cases. *Diagn Cytopathol* 1998;18(6):462-467.
78. Feichter GE, Haberthur F, Gobat S, Dalquen P: Breast cytology. Statistical analysis and cytohistologic correlations. *Acta Cytol* 1997;41(2):327-332.
79. Kanchanabat B, Kanchanapitak P, Thanapongsathorn W, Manomaiphiboon A: Fine-needle aspiration cytology for diagnosis and management of palpable breast mass. *Aust N Z J Surg* 2000;70(11):791-794.

80. Smith SD, Cason Z, Cabaniss DE, et al.: Accuracy of fine needle aspiration biopsy of the breast. *Biomed Sci Instrum* 1997;33:286-291.
81. Shah SH, Kayani N, Hasan SH, et al.: Diagnostic evaluation of fine needle aspiration cytology in the management of palpable breast lesions. *J Pak Med Assoc* 1998;48(1):7-8.
82. Vetrani A, Fulciniti F, Di Benedetto G, et al.: Fine-needle aspiration biopsies of breast masses. An additional experience with 1153 cases (1985 to 1988) and a meta-analysis. *Cancer* 1992;69(3):736-740.
83. Zarbo RJ, Howanitz PJ, Bachner P: Interinstitutional comparison of performance in breast fine-needle aspiration cytology. A Q-probe quality indicator study. *Arch Pathol Lab Med* 1991;115(8):743-750.
84. Kim A, Lee J, Choi JS, et al.: Fine needle aspiration cytology of the breast. Experience at an outpatient breast clinic. *Acta Cytol* 2000;44(3):361-367.
85. Florentine BD, Staymates B, Rabadi M, et al.: The reliability of fine-needle aspiration biopsy as the initial diagnostic procedure for palpable masses: A 4-year experience of 730 patients from a community hospital-based outpatient aspiration biopsy clinic. *Cancer* 2006;107(2):406-416.
86. Feldman PSaC, JL, (ed) *Fine Needle Aspiration Cytology and Its Clinical Applications: Breast and Lung*. Chicago, ASCP Press, 1985.
87. Azavedo E, Svane G, Auer G: Stereotactic fine-needle biopsy in 2594 mammographically detected non-palpable lesions. *Lancet* 1989;1(8646):1033-1036.
88. Bibbo M, Scheiber M, Cajulis R, et al.: Stereotaxic fine needle aspiration cytology of clinically occult malignant and premalignant breast lesions. *Acta Cytol* 1988;32(2):193-201.
89. Evans WP, Cade SH: Needle localization and fine-needle aspiration biopsy of nonpalpable breast lesions with use of standard and stereotactic equipment. *Radiology* 1989;173(1):53-56.
90. Hann L, Ducatman BS, Wang HH, et al.: Nonpalpable breast lesions: evaluation by means of fine-needle aspiration cytology. *Radiology* 1989;171(2):373-376.
91. Masood S: Occult breast lesions and aspiration biopsy: a new challenge. *Diagn Cytopathol* 1993;9(6):613-614.
92. Ciatto S, Rosselli Del Turco M, Ambrogetti D, et al.: Solid nonpalpable breast lesions: Success and failure of guided fine-needle aspiration cytology in a consecutive series of 2444 cases. *Acta Radiol* 1997;38(5):815-820.
93. Klijanienko J, Cote JE, Thibault F, et al.: Ultrasound-guided fine-needle aspiration cytology of nonpalpable breast lesions: Institut Curie's experience with 198 histologically correlated cases. *Cancer* 1998;84(1):36-41.
94. Zancanati F, Bonifacio D, Falconieri G, Di Bonito L: Role of fine-needle aspiration cytology in nonpalpable mammary lesions: A comparative cytohistologic study based on 308 cases. *Diagn Cytopathol* 2000;23(2):87-91.
95. Buchbinder SS, Gurell DS, Tarlow MM, et al.: Role of US-guided fine-needle aspiration with on-site cytopathologic evaluation in management of nonpalpable breast lesions. *Acad Radiol* 2001;8(4):322-327.
96. Hatada T, Aoki I, Okada K, et al.: Usefulness of ultrasound-guided, fine-needle aspiration biopsy for palpable breast tumors. *Arch Surg* 1996;131(10):1095-1098.
97. Saarela AO, Kiviniemi HO, Rissanen TJ, Paloneva TK: Nonpalpable breast lesions: Pathologic correlation of ultrasonographically guided fine-needle aspiration biopsy. *J Ultrasound Med* 1996;15(8):549-553; quiz 55-56.
98. Sarfati MR, Fox KA, Warneke JA, et al.: Stereotactic fine-needle aspiration cytology of nonpalpable breast lesions: An analysis of 258 consecutive aspirates. *Am J Surg* 1994;168(6):529-531; discussion 531-532.
99. Sneige N, Singletary SE: Fine-needle aspiration of the breast: Diagnostic problems and approaches to surgical management. *Pathol Annu* 1994;29(Pt 1):281-301.
100. Cote JE, Klijanienko J, Meunier M, et al.: Stereotactic fine-needle aspiration cytology of nonpalpable breast lesions: Institut Curie's experience of 243 histologically correlated cases. *Cancer* 1998;84(2):77-83.
101. Okamoto H, Ogawara T, Inoue S, et al.: Clinical management of nonpalpable or small breast masses by fine-needle aspiration biopsy (FNAB) under ultrasound guidance. *J Surg Oncol* 1998;67(4):246-250.
102. Cangiarella J, Mercado CL, Symmans WE, et al.: Stereotaxic aspiration biopsy in the evaluation of mammographically detected clustered microcalcification. *Cancer* 1998;84(4):226-230.
103. Tanaka K, Shoji T, Tominaga Y, et al.: Statistical analysis of diagnostic failure of fine needle aspiration cytology (FNAC) in breast cancer. *J Surg Oncol* 2001;76(2):100-105.
104. Barrows GH, Anderson TJ, Lamb JL, Dixon JM: Fine-needle aspiration of breast cancer. Relationship of clinical factors to cytology results in 689 primary malignancies. *Cancer* 1986;58(7):1493-1498.
105. Palombini L, Fulciniti F, Vetrani A, et al.: Fine-needle aspiration biopsies of breast masses A critical analysis of 1956 cases in 8 years (1976-1984). *Cancer* 1988;61(11):2273-2277.
106. Rocha PD, Nadkarni NS, Menezes S: Fine needle aspiration biopsy of breast lesions and histopathologic correlation An analysis of 837 cases in four years. *Acta Cytol* 1997;41(3):705-712.
107. Dray M, Mayall F, Darlington A: Improved fine needle aspiration (FNA) cytology results with a near patient diagnosis service for breast lesions. *Cytopathology* 2000;11(1):32-37.
108. Ljung BM, Drejet A, Chiampi N, et al.: Diagnostic accuracy of fine-needle aspiration biopsy is determined by physician training in sampling technique. *Cancer* 2001;93(4):263-268.
109. Mayall F, Denford A, Chang B, Darlington A: Improved FNA cytology results with a near patient diagnosis service for non-breast lesions. *J Clin Pathol* 1998;51(7):541-544.
110. Cohen MB, Rodgers RP, Hales MS, et al.: Influence of training and experience in fine-needle aspiration biopsy of breast. Receiver operating characteristics curve analysis. *Arch Pathol Lab Med* 1987;111(6):518-520.
111. Lee KR, Foster RS, Papillo JL: Fine needle aspiration of the breast. Importance of the aspirator. *Acta Cytol* 1987;31(3):281-284.
112. Harton AM, Wang HH, Schnitt SJ, Jacobs TW: p63 Immunocytochemistry improves accuracy of diagnosis with fine-needle aspiration of the breast. *Am J Clin Pathol* 2007;128(1):80-85.
113. Ciatto S, Bonardi R, Cariaggi MP: Performance of fine-needle aspiration cytology of the breast—Multicenter study of 23,063 aspirates in ten Italian laboratories. *Tumori* 1995;81(1):13-17.
114. Wang HH, Ducatman BS: Fine needle aspiration of the breast. A probabilistic approach to diagnosis of carcinoma. *Acta Cytol* 1998;42(2):285-289.
115. Rollins SD: Criteria for cytologic reporting of breast fine needle aspiration. *Acta Cytol* 1998;42(6):1482-1483.
116. Boerner S, Fornage BD, Singletary E, Sneige N: Ultrasound-guided fine-needle aspiration (FNA) of nonpalpable breast lesions: a review of 1885 FNA cases using the National Cancer Institute—Supported recommendations on the uniform approach to breast FNA. *Cancer* 1999;87(1):19-24.
117. Howell LP: Equivocal diagnoses in breast aspiration biopsy cytology: Sources of uncertainty and the role of "atypical/indeterminate" terminology. *Diagn Cytopathol* 1999;21(3):217-222.
118. Ayata G, Abu-Jawdeh GM, Fraser JL, et al.: Accuracy and consistency in application of a probabilistic approach

- to reporting breast fine needle aspiration. *Acta Cytol* 2003;47(6):973-978.
119. Sidawy MK, Stoler MH, Frable WJ, et al.: Interobserver variability in the classification of proliferative breast lesions by fine-needle aspiration: Results of the Papanicolaou Society of Cytopathology Study. *Diagn Cytopathol* 1998;18(2):150-165.
 120. Page DL, Johnson JE, Dupont WD: Probabilistic approach to the reporting of fine-needle aspiration cytology of the breast. *Cancer* 1997;81(1):6-9.
 121. Howell LP, Lin-Chang L: Cytomorphology of common malignant tumors of the breast. *Clin Lab Med* 2005;25(4):733-760, vii.
 122. Gornstein B, Jacobs T, Bedard Y, et al.: Interobserver agreement of a probabilistic approach to reporting breast fine-needle aspirations on ThinPrep. *Diagn Cytopathol* 2004;30(6):389-395.
 123. Layfield LJ, Mooney EE, Glasgow B, et al.: What constitutes an adequate smear in fine-needle aspiration cytology of the breast? *Cancer* 1997;81(1):16-21.
 124. Boerner S, Sneige N: Specimen adequacy and false-negative diagnosis rate in fine-needle aspirates of palpable breast masses. *Cancer* 1998;84(6):344-348.
 125. Salami N, Hirschowitz SL, Nieberg RK, Apple SK: Triple test approach to inadequate fine needle aspiration biopsies of palpable breast lesions. *Acta Cytol* 1999;43(3):339-343.
 126. Lee HC, Ooi PJ, Poh WT, Wong CY: Impact of inadequate fine-needle aspiration cytology on outcome of patients with palpable breast lesions. *Aust N Z J Surg* 2000;70(9):656-659.
 127. Lau SK, McKee GT, Weir MM, et al.: The negative predicative value of breast fine-needle aspiration biopsy: The Massachusetts General Hospital experience. *Breast J* 2004;10(6):487-491.
 128. Lim JC, Al-Masri H, Salhadar A, et al.: The significance of the diagnosis of atypia in breast fine-needle aspiration. *Diagn Cytopathol* 2004;31(5):285-288.
 129. El Aouni N, Laurent I, Terrier P, et al.: Granular cell tumor of the breast. *Diagn Cytopathol* 2007;35(11):725-727.
 130. Watanabe K, Nomura M, Hashimoto Y, et al.: Fine-needle aspiration cytology of apocrine adenosis of the breast: Report on three cases. *Diagn Cytopathol* 2007;35(5):296-299.
 131. Schnitt SJ, Connolly JL, Tavassoli FA, et al.: Interobserver reproducibility in the diagnosis of ductal proliferative breast lesions using standardized criteria. *Am J Surg Pathol* 1992;16(12):1133-1143.
 132. Midulla C, Cenci M, De Iorio P, et al.: The value of fine needle aspiration cytology in the diagnosis of breast proliferative lesions. *Anticancer Res* 1995;15(6B):2619-2622.
 133. McKee GT, Tildsley G, Hammond S: Cytologic diagnosis and grading of ductal carcinoma in situ. *Cancer* 1999;87(4):203-209.
 134. Malamud YR, Ducatman BS, Wang HH: Comparative features of comedo and noncomedo ductal carcinoma in situ of the breast on fine-needle aspiration biopsy. *Diagn Cytopathol* 1992;8(6):571-576.
 135. Abendroth CS, Wang HH, Ducatman BS: Comparative features of carcinoma in situ and atypical ductal hyperplasia of the breast on fine-needle aspiration biopsy specimens. *Am J Clin Pathol* 1991;96(5):654-659.
 136. Stanley MW, Henry-Stanley MJ, Zera R: Atypia in breast fine-needle aspiration smears correlates poorly with the presence of a prognostically significant proliferative lesion of ductal epithelium. *Hum Pathol* 1993;24(6):630-635.
 137. Silverman JF, Masood S, Ducatman BS, et al.: Can FNA biopsy separate atypical hyperplasia, carcinoma in situ, and invasive carcinoma of the breast? Cytomorphologic criteria and limitations in diagnosis. *Diagn Cytopathol* 1993;9(6):713-728.
 138. al-Kaisi N: The spectrum of the "gray zone" in breast cytology: A review of 186 cases of atypical and suspicious cytology. *Acta Cytol* 1994;38(6):898-908.
 139. King EB, Chew KL, Hom JD, et al.: Characterization by image cytometry of duct epithelial proliferative disease of the breast. *Mod Pathol* 1991;4(3):291-296.
 140. Norris HJ, Bahr GF, Mikel UV: A comparative morphometric and cytophotometric study of intraductal hyperplasia and intraductal carcinoma of the breast. *Anal Quant Cytol Histol* 1988;10(1):1-9.
 141. Komoike Y, Motomura K, Inaji H, Koyama H: Diagnosis of ductal carcinoma in situ (DCIS) and intraductal papilloma using fluorescence in situ hybridization (FISH) analysis. *Breast Cancer* 2000;7(4):332-336.
 142. Istvanic S, Fischer AH, Banner BF, et al.: Cell blocks of breast FNAs frequently allow diagnosis of invasion or histological classification of proliferative changes. *Diagn Cytopathol* 2007;35(5):263-269.
 143. Bottles K, Chan JS, Holly EA, et al.: Cytologic criteria for fibroadenoma. A step-wise logistic regression analysis. *Am J Clin Pathol* 1988;89(6):707-713.
 144. Dejmek A, Lindholm K: Frequency of cytologic features in fine needle aspirates from histologically and cytologically diagnosed fibroadenomas. *Acta Cytol* 1991;35(6):695-699.
 145. Krishnamurthy S, Ashfaq R, Shin HJ, Sneige N: Distinction of phyllodes tumor from fibroadenoma: A reappraisal of an old problem. *Cancer* 2000;90(6):342-349.
 146. Veneti S, Manek S: Benign phyllodes tumour vs. fibroadenoma: FNA cytological differentiation. *Cytopathology* 2001;12(5):321-328.
 147. Stanley MW, Tani EM, Skoog L: Fine-needle aspiration of fibroadenomas of the breast with atypia: A spectrum including cases that cytologically mimic carcinoma. *Diagn Cytopathol* 1990;6(6):375-382.
 148. Mulford DK, Dawson AE: Atypia in fine needle aspiration cytology of nonpalpable and palpable mammographically detected breast lesions. *Acta Cytol* 1994;38(1):9-17.
 149. Kollur SM, El Hag IA: FNA of breast fibroadenoma: Observer variability and review of cytomorphology with cytohistological correlation. *Cytopathology* 2006;17(5):239-244.
 150. Rogers LA, Lee KR: Breast carcinoma simulating fibroadenoma or fibrocystic change by fine-needle aspiration. A study of 16 cases. *Am J Clin Pathol* 1992;98(2):155-160.
 151. Lopez-Ferrer P, Jimenez-Heffernan JA, Vicandi B, et al.: Fine needle aspiration cytology of breast fibroadenoma: A cytohistologic correlation study of 405 cases. *Acta Cytol* 1999;43(4):579-586.
 152. Myers T, Wang HH: Fibroadenoma mimicking papillary carcinoma on ThinPrep of fine-needle aspiration of the breast. *Arch Pathol Lab Med* 2000;124(11):1667-1669.
 153. Vesoulis Z, Kashkari S: Fine needle aspiration of secretory breast carcinoma resembling lactational changes: A case report. *Acta Cytol* 1998;42(4):1032-1036.
 154. Grenko RT, Lee KP, Lee KR: Fine needle aspiration cytology of lactating adenoma of the breast: A comparative light microscopic and morphometric study. *Acta Cytol* 1990;34(1):21-26.
 155. Siddiqui MT, Zakowski MF, Ashfaq R, Ali SZ: Breast masses in males: Multi-institutional experience on fine-needle aspiration. *Diagn Cytopathol* 2002;26(2):87-91.
 156. Dodd LG, Sneige N, Reece GP, Fornage B: Fine-needle aspiration cytology of silicone granulomas in the augmented breast. *Diagn Cytopathol* 1993;9(5):498-502.
 157. Balco MT, Ali SZ: Asteroid bodies in silicone lymphadenitis on fine-needle aspiration. *Diagn Cytopathol* 2007;35(11):715-716.
 158. Ku NN, Mela NJ, Fiorica JV, et al.: Role of fine needle aspiration cytology after lumpectomy. *Acta Cytol* 1994;38(6):927-932.
 159. Dornfeld JM, Thompson SK, Shurbaji MS: Radiation-induced changes in the breast: A potential diagnostic pitfall on fine-needle aspiration. *Diagn Cytopathol* 1992;8(1):79-80; discussion 81.

160. Peterse JL, Thunnissen FB, van Heerde P: Fine needle aspiration cytology of radiation-induced changes in nonneoplastic breast lesions: Possible pitfalls in cytodiagnosis. *Acta Cytol* 1989;33(2):176-180.
161. Gupta RK: Radiation-induced cellular changes in the breast: A potential diagnostic pitfall in fine needle aspiration cytology. *Acta Cytol* 1989;33(1):141-142.
162. Hirata S, Saito T, Kiyanagi K, et al.: Granulomatous mastitis diagnosed by core-needle biopsy and successfully treated with corticosteroid therapy: A case report. *Breast Cancer* 2003;10(4):378-381.
163. Martinez-Parra D, Nevado-Santos M, Melendez-Guerrero B, et al.: Utility of fine-needle aspiration in the diagnosis of granulomatous lesions of the breast. *Diagn Cytopathol* 1997;17(2):108-114.
164. Poniecka AW, Krasuski P, Gal E, et al.: Granulomatous inflammation of the breast in a pregnant woman: Report of a case with fine needle aspiration diagnosis. *Acta Cytol* 2001;45(5):797-801.
165. Silverman JF, Lannin DR, Unverferth M, Norris HT: Fine needle aspiration cytology of subareolar abscess of the breast. Spectrum of cytomorphologic findings and potential diagnostic pitfalls. *Acta Cytol* 1986;30(4):413-419.
166. Kapila K, Verma K: Cytomorphological spectrum in gynecomastia: A study of 389 cases. *Cytopathology* 2002; 13(5):300-308.
167. Sneige N, Holder PD, Katz RL, et al.: Fine-needle aspiration cytology of the male breast in a cancer center. *Diagn Cytopathol* 1993;9(6):691-697.
168. Vetto J, Schmidt W, Pommier R, et al.: Accurate and cost-effective evaluation of breast masses in males. *Am J Surg* 1998;175(5):383-387.
169. Kumar PV, Talei AR, Malekhusseini SA, et al.: Papillary carcinoma of the breast. Cytologic study of nine cases. *Acta Cytol* 1999;43(5):767-770.
170. Saad RS, Kanbour-Shakir A, Syed A, Kanbour A: Sclerosing papillary lesion of the breast: A diagnostic pitfall for malignancy in fine needle aspiration biopsy. *Diagn Cytopathol* 2006;34(2):114-118.
171. Usami S, Moriya T, Kasajima A, et al.: Pathological aspects of core needle biopsy for non-palpable breast lesions. *Breast Cancer* 2005;12(4):272-278.
172. Nayar R, De Frias DV, Bourtsos EP, et al.: Cytologic differential diagnosis of papillary pattern in breast aspirates: correlation with histology. *Ann Diagn Pathol* 2001;5(1):34-42.
173. Tavassoli FA, Devilee P: Tumours of the Breast and Female Genital Organs. Lyon, France, IARC Press, 2003.
174. Bhattarai S, Kapila K, Verma K: Phyllodes tumor of the breast. A cytohistologic study of 80 cases. *Acta Cytol* 2000; 44(5):790-796.
175. Shabalova IP, Chemeris GJ, Ermilova VD, et al.: Phyllodes tumour: Cytologic and histologic presentation of 22 cases, and immunohistochemical demonstration of p53. *Cytopathology* 1997;8(3):177-187.
176. Shabb NS: Phyllodes tumor. Fine needle aspiration cytology of eight cases. *Acta Cytol* 1997;41(2):321-326.
177. Rao CR, Narasimhamurthy NK, Jaganathan K, et al.: Cystosarcoma phyllodes Diagnosis by fine needle aspiration cytology. *Acta Cytol* 1992;36(2):203-207.
178. Jayaram G, Sthaneshwar P: Fine-needle aspiration cytology of phyllodes tumors. *Diagn Cytopathol* 2002;26(4):222-227.
179. Florentine BD, Cobb CJ, Frankel K, et al.: Core needle biopsy. A useful adjunct to fine-needle aspiration in select patients with palpable breast lesions. *Cancer* 1997;81(1):33-39.
180. Dusenbery D, Frable WJ: Fine needle aspiration cytology of phyllodes tumor. Potential diagnostic pitfalls. *Acta Cytol* 1992;36(2):215-221.
181. Stanley MW, Tani EM, Rutqvist LE, Skoog L: Cystosarcoma phyllodes of the breast: A cytologic and clinicopathologic study of 23 cases. *Diagn Cytopathol* 1989;5(1):29-34.
182. Silverman JF, Geisinger KR, Frable WJ: Fine-needle aspiration cytology of mesenchymal tumors of the breast. *Diagn Cytopathol* 1988;4(1):50-58.
183. Desai S, Krishnamurthy S: Stromal fragments in invasive carcinoma. Source of diagnostic difficulty in aspiration cytology. *Acta Cytol* 1997;41(6):1747-1750.
184. Chhieng DC, Cangiarella JF, Waisman J, et al.: Fine-needle aspiration cytology of spindle cell lesions of the breast. *Cancer* 1999;87(6):359-371.
185. Smigal C, Jemal A, Ward E, et al.: Trends in breast cancer by race and ethnicity: Update 2006. *CA Cancer J Clin* 2006;56(3):168-183.
186. Ducatman BS, Emery ST, Wang HH: Correlation of histologic grade of breast carcinoma with cytologic features on fine-needle aspiration of the breast. *Mod Pathol* 1993;6(5):539-543.
187. Garg S, Mohan H, Bal A, et al.: A comparative analysis of core needle biopsy and fine-needle aspiration cytology in the evaluation of palpable and mammographically detected suspicious breast lesions. *Diagn Cytopathol* 2007;35(11):681-689.
188. Park IA, Ham EK: Fine needle aspiration cytology of palpable breast lesions Histologic subtype in false negative cases. *Acta Cytol* 1997;41(4):1131-1138.
189. Sneige N, White VA, Katz RL, et al.: Ductal carcinoma-in-situ of the breast: fine-needle aspiration cytology of 12 cases. *Diagn Cytopathol* 1989;5(4):371-377.
190. Wang HH, Ducatman BS, Eick D: Comparative features of ductal carcinoma in situ and infiltrating ductal carcinoma of the breast on fine-needle aspiration biopsy. *Am J Clin Pathol* 1989;92(6):736-740.
191. McKee GT, Tambouret RH, Finkelstein D: Fine-needle aspiration cytology of the breast: Invasive vs. in situ carcinoma. *Diagn Cytopathol* 2001;25(1):73-77.
192. Klijanienko J, Katsahian S, Vielh P, Masood S: Stromal infiltration as a predictor of tumor invasion in breast fine-needle aspiration biopsy. *Diagn Cytopathol* 2004;30(3):182-186.
193. Maygarden SJ, Brock MS, Novotny DB: Are epithelial cells in fat or connective tissue a reliable indicator of tumor invasion in fine-needle aspiration of the breast? *Diagn Cytopathol* 1997;16(2):137-142.
194. Bonzanini M, Gilioli E, Brancato B, et al.: The cytopathology of ductal carcinoma in situ of the breast. A detailed analysis of fine needle aspiration cytology of 58 cases compared with 101 invasive ductal carcinomas. *Cytopathology* 2001;12(2): 107-119.
195. Bondeson L, Lindholm K: Prediction of invasiveness by aspiration cytology applied to nonpalpable breast carcinoma and tested in 300 cases. *Diagn Cytopathol* 1997; 17(5):315-320.
196. Benoit JL, Kara R, McGregor SE, Duggan MA: Fibroadenoma of the breast: diagnostic pitfalls of fine-needle aspiration. *Diagn Cytopathol* 1992;8(6):643-647; discussion 647-648.
197. Cariaggi MP, Bulgaresi P, Confortini M, et al.: Analysis of the causes of false negative cytology reports on breast cancer fine needle aspirates. *Cytopathology* 1995;6(3):156-161.
198. Nijhawan R, Rajwanshi A: Cytomorphologic and morphometric limitations of the assessment of atypia in fibroadenoma of the breast. *Anal Quant Cytol Histol* 2005;27(5):273-276.
199. Yokoyama T, Kawahara A, Kage M, et al.: Image analysis of irregularity of cluster shape in cytological diagnosis of breast tumors: Cluster analysis with 2D-fractal dimension. *Diagn Cytopathol* 2005;33(2):71-77.
200. Sturgis CD, Sethi S, Cajulis RS, et al.: Diagnostic significance of "benign pairs" and signet ring cells in fine needle aspirates (FNAs) of the breast. *Cytopathology* 1998;9(5):308-319.

201. Prasad ML, Hyjek E, Giri DD, et al.: Double immunolabeling with cytokeratin and smooth-muscle actin in confirming early invasive carcinoma of breast. *Am J Surg Pathol* 1999;23(2):176-181.
202. Dey P, Luthra UK: False negative cytologic diagnosis of breast carcinoma. *Acta Cytol* 1999;43(5):801-805.
203. Ayata G, Wang HH: Fine needle aspiration cytology of lobular carcinoma in situ on ThinPrep. *Diagn Cytopathol* 2005;32(5):276-280.
204. Levine PH, Waisman J, Yang GC: Aspiration cytology of cystic carcinoma of the breast. *Diagn Cytopathol* 2003;28(1):39-44.
205. Layfield LJ, Glasgow BJ, Hirschowitz S, Dodd LG: Intramammary lymph nodes: Cytologic findings and implications for fine-needle aspiration cytology diagnosis of breast nodules. *Diagn Cytopathol* 1997;17(3):223-229.
206. Kleer CG, Michael CW: Fine-needle aspiration of breast carcinomas with prominent lymphocytic infiltrate. *Diagn Cytopathol* 2000;23(1):39-42.
207. Lam WW, Chu WC, Tse GM, et al.: Role of fine needle aspiration and tru cut biopsy in diagnosis of mucinous carcinoma of breast—from a radiologist's perspective. *Clin Imaging* 2006;30(1):6-10.
208. Dawson AE, Mulford DK: Fine needle aspiration of mucinous (colloid) breast carcinoma Nuclear grading and mammographic and cytologic findings. *Acta Cytol* 1998;42(3):668-672.
209. Stanley MW, Tani EM, Skoog L: Mucinous breast carcinoma and mixed mucinous-infiltrating ductal carcinoma: A comparative cytologic study. *Diagn Cytopathol* 1989;5(2):134-138.
210. Jayaram G, Swain M, Chew MT et al.: Cytology of mucinous carcinoma of breast: A report of 28 cases with histological correlation. *Malays J Pathol* 2000;22(2):65-71.
211. Pillai KR, Jayasree K, Jayalal KS, et al.: Mucinous carcinoma of breast with abundant psammoma bodies in fine-needle aspiration cytology: A case report. *Diagn Cytopathol* 2007;35(4):230-233.
212. Wong NL, Wan SK: Comparative cytology of mucocoelelike lesion and mucinous carcinoma of the breast in fine needle aspiration. *Acta Cytol* 2000;44(5):765-770.
213. de la Vega M, Rey A, Afonso JL: Fine needle aspiration of mucocoelelike lesions: differential diagnosis with colloid carcinoma. *Acta Cytol* 1998;42(3):832-833.
214. Simsir A, Tsang P, Greenebaum E: Additional mimics of mucinous mammary carcinoma: Fibroepithelial lesions. *Am J Clin Pathol* 1998;109(2):169-172.
215. Mitnick JS, Gianutsos R, Pollack AH, et al.: Tubular carcinoma of the breast: Sensitivity of diagnostic techniques and correlation with histopathology. *AJR Am J Roentgenol* 1999;172(2):319-323.
216. Dawson AE, Logan-Young W, Mulford DK: Aspiration cytology of tubular carcinoma: Diagnostic features with mammographic correlation. *Am J Clin Pathol* 1994;101(4):488-492.
217. Cangiarella J, Waisman J, Shapiro RL, Simsir A: Cytologic features of tubular adenocarcinoma of the breast by aspiration biopsy. *Diagn Cytopathol* 2001;25(5):311-315.
218. Bondeson L, Lindholm K: Aspiration cytology of tubular breast carcinoma. *Acta Cytol* 1990;34(1):15-20.
219. Kato T, Tohnosu N, Suwa T, et al.: Metaplastic breast carcinoma with chondrosarcomatous differentiation: Fine-needle aspiration cytology findings: A case report. *Diagn Cytopathol* 2006;34(11):772-775.
220. Yen H, Florentine B, Kelly LK, et al.: Fine-needle aspiration of a metaplastic breast carcinoma with extensive melanocytic differentiation: A case report. *Diagn Cytopathol* 2000;23(1):46-50.
221. Gupta RK: Cytodiagnostic patterns of metaplastic breast carcinoma in aspiration samples: A study of 14 cases. *Diagn Cytopathol* 1999;20(1):10-12.
222. Nogueira M, Andre S, Mendonca E: Metaplastic carcinomas of the breast—fine needle aspiration (FNA) cytology findings. *Cytopathology* 1998;9(5):291-300.
223. Straathof D, Yakimets WW, Mourad WA. Fine-needle aspiration cytology of sarcomatoid carcinoma of the breast: A cytologically overlooked neoplasm. *Diagn Cytopathol* 1997;16(3):242-246.
224. Castella E, Gomez-Plaza MC, Urban A, Llatjos M: Fine-needle aspiration biopsy of metaplastic carcinoma of the breast: Report of a case with abundant myxoid ground substance. *Diagn Cytopathol* 1996;14(4):325-327.
225. Saad RS, Silverman JF, Julian T, et al.: Atypical squamous metaplasia of seromas in breast needle aspirates from irradiated lumpectomy sites: a potential pitfall for false-positive diagnoses of carcinoma. *Diagn Cytopathol* 2002;26(2):104-108.
226. Jun Wei X, Hiotis K, Garcia R, Hummel Levine P: Leiomyosarcoma of the breast: A difficult diagnosis on fine-needle aspiration biopsy. *Diagn Cytopathol* 2003;29(3):172-178.
227. Jaffer S, Emanuel P, Burstein D, Goldfarb A: Cytologic findings of spindle cell ductal carcinoma in situ of the breast: A case report. *Acta Cytol* 2005;49(3):323-326.
228. Gherardi G, Rossi S, Perrone S, Scanni A: Angiosarcoma after breast-conserving therapy: Fine-needle aspiration biopsy, immunocytochemistry, and clinicopathologic correlates. *Cancer* 2005;105(3):145-151.
229. Gupta RK, McHutchison AG, Simpson JS, Dowle CS: Fine needle aspiration cytodiagnosis of apocrine carcinoma of the breast. *Cytopathology* 1992;3(5):321-326.
230. Ro JY, Ngadiman S, Sahin A, et al.: Intraluminal crystalloids in breast carcinoma. Immunohistochemical, ultrastructural, and energy-dispersive x-ray element analysis in four cases. *Arch Pathol Lab Med* 1997;121(6):593-598.
231. Stahlschmidt J, Liston J, Aslam MM, Carder PJ: Fine needle aspiration cytology of adenoid cystic carcinoma of the breast. *Cytopathology* 2001;12(4):266-269.
232. Tsuchiya A, Nozawa Y, Watanabe T, et al.: Adenoid cystic carcinoma of the breast: report of a case. *Surg Today* 2000;30(7):655-657.
233. Gupta RK, Green C, Naran S, et al.: Fine-needle aspiration cytology of adenoid cystic carcinoma of the breast. *Diagn Cytopathol* 1999;20(2):82-84.
234. Culubret M, Roig I: Fine-needle aspiration biopsy of adenoid cystic carcinoma of the breast: A case report. *Diagn Cytopathol* 1996;15(5):431-434.
235. Gupta RK, Dowle C: Fine-needle aspiration cytodiagnosis of adenoid cystic carcinoma of the breast. *Diagn Cytopathol* 1996;14(4):328-330.
236. Saqi A, Mercado CL, Hamele-Bena D: Adenoid cystic carcinoma of the breast diagnosed by fine-needle aspiration. *Diagn Cytopathol* 2004;30(4):271-274.
237. Quinodoz IS, Berger SD, Schafer P, Remadi S: Adenoid cystic carcinoma of the breast: Utility of immunocytochemical study with collagen IV on fine-needle aspiration. *Diagn Cytopathol* 1997;16(5):442-445.
238. Mastropasqua MG, Maiorano E, Pruneri G, et al.: Immunoreactivity for c-kit and p63 as an adjunct in the diagnosis of adenoid cystic carcinoma of the breast. *Mod Pathol* 2005;18(10):1277-1282.
239. Sola Perez J, Perez-Guillermo M, Bas Bernal A, Rodriguez Bermejo M: Diagnosis of collagenous spherulosis of the breast by fine needle aspiration cytology. A report of two cases. *Acta Cytol* 1993;37(5):725-728.

240. Highland KE, Finley JL, Neill JS, Silverman JF: Collagenous spherulosis. Report of a case with diagnosis by fine needle aspiration biopsy with immunocytochemical and ultrastructural observations. *Acta Cytol* 1993;37(1):3-9.
241. McCluggage WG, McManus DI, Caughley LM: Fine needle aspiration (FNA) cytology of adenoid cystic carcinoma and adenomyoepithelioma of breast: Two lesions rich in myoepithelial cells. *Cytopathology* 1997;8(1):31-39.
242. Lee WY: Fine needle aspiration cytology of adenomyoepithelioma of the breast: A case indistinguishable from phyllodes tumor in cytologic findings and clinical behavior. *Acta Cytol* 2000;44(3):488-490.
243. Ellis IO, Pinder SE, Lee AHS: Tumors of the breast. In Fletcher CDM (ed): *Diagnostic Histopathology of Tumors*, 3rd ed. London, Churchill Livingstone Elsevier, 2007.
244. Levine PH, Zamuco R, Yee HT: Role of fine-needle aspiration cytology in breast lymphoma. *Diagn Cytopathol* 2004;30(5):332-340.
245. Kumar PV, Vasei M, Daneshbod Y, et al.: Breast myeloma: A report of 3 cases with fine needle aspiration cytologic findings. *Acta Cytol* 2005;49(4):445-448.
246. Vetto JT, Beer TM, Fidda N, et al.: Fine-needle aspiration diagnosis of plasmacytoma presenting as breast masses in a patient on estrogen therapy for prostate cancer. *Diagn Cytopathol* 2004;31(6):417-419.
247. Munitiz V, Rios A, Canovas J, et al.: Primitive leiomyosarcoma of the breast: Case report and review of the literature. *Breast* 2004;13(1):72-76.
248. Kiyozuka Y, Koyama H, Nakata M, et al.: Diagnostic cytopathology in type II angiosarcoma of the breast: a case report. *Acta Cytol* 2005;49(5):560-566.
249. Tamura G, Sasou S, Kudoh S, et al.: Primitive neuroectodermal tumor of the breast: Immunohistochemistry and fluorescence in situ hybridization. *Pathol Int* 2007;57(8):509-512.
250. Trihita H, Valavanis C, Markidou S, et al.: Primary osteogenic sarcoma of the breast: Cytomorphologic study of 3 cases with histologic correlation. *Acta Cytol* 2007;51(3):443-450.
251. Ewing CA, Miller MJ, Chhieng D, Lin O: Nonepithelial malignancies mimicking primary carcinoma of the breast. *Diagn Cytopathol* 2004;31(5):352-357.

Thyroid

EDMUND S. CIBAS

ASPIRATION TECHNIQUE AND SLIDE PREPARATION

TERMINOLOGY FOR REPORTING RESULTS

ACCURACY

EVALUATION OF THE SPECIMEN

BENIGN CONDITIONS

Benign Follicular Nodules
Chronic Lymphocytic (Hashimoto) Thyroiditis
Subacute (de Quervain) Thyroiditis
Riedel Disease
Amyloid Goiter
Black Thyroid
Radiation Changes

ATYPICAL CELLS OF UNDETERMINED SIGNIFICANCE

SUSPICIOUS FOR A FOLLICULAR NEOPLASM

SUSPICIOUS FOR A HÜRTHLE CELL NEOPLASM

MALIGNANT CONDITIONS

Papillary Carcinoma
Poorly Differentiated Carcinoma
Undifferentiated (Anaplastic) Carcinoma
Squamous Cell Carcinoma
Medullary Thyroid Carcinoma
Lymphoma
Metastatic Carcinoma

PARATHYROID TUMORS

The main indication for fine-needle aspiration (FNA) of the thyroid is a thyroid nodule, one or more of which are detected by palpation in 4% to 7% of adults.^{1,2} The prevalence of thyroid nodules is significantly higher (20% to 30%, in some studies as high as 70% of adults) when nonpalpable nodules are included, like those detected by ultrasonography or at autopsy.³⁻⁶

Although a thyroid nodule raises the suspicion of cancer, fewer than 5% are malignant.^{4,7} Given the high prevalence of nodules, combined with the impracticality of surgically excising all nodules, FNA plays a vital role as a screening test. Few cytology tests have so effectively decreased unnecessary surgery while increasing the yield of malignancy.⁸⁻¹⁰ At the Mayo Clinic the percentage of patients requiring thyroid surgery dropped from 67% to 43% 1 year after FNA was introduced (around 1980); the percentage of excised nodules that were malignant rose from 14% to 29%; and the cost of a workup of a thyroid nodule decreased by 25%.⁹ Since then, the reduction in unnecessary surgery has been even greater—the percentage of excised nodules that are malignant is now 45% to 56%.^{11,12}

Every patient with a palpable thyroid nodule is a candidate for FNA. Palpation is not always accurate in assessing the presence and extent of thyroid nodularity, however. After ultrasound (US) examination, 20% of patients with a palpable thyroid nodule prove not to have a nodule greater than 1 cm, and conversely, additional significant nodules are often

found that were not appreciated by palpation.¹³ For this reason, a thyroid US is often obtained before the FNA to confirm that the palpated nodule meets biopsy criteria.¹⁴

FNA can be avoided in patients who have a hyperfunctioning (“hot”) nodule (about 5% of all nodules) by also obtaining a serum thyrotropin level.¹⁴ If the thyroid-stimulating hormone (TSH) level is normal or elevated, an FNA is usually performed. But if the TSH level is depressed, a radionuclide thyroid scan is obtained, and if the scan confirms that the nodule is indeed hot, an FNA is not indicated because a hot thyroid nodule is rarely malignant.^{15,16}

An increasing number of thyroid nodules are being detected incidentally by imaging studies of the neck (thyroid “incidentalomas”). The various imaging modalities include US (for carotid artery disease), sestamibi scans (for hyperparathyroidism), computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). It is not practical or advisable to perform an FNA on all incidentalomas. Evidence suggests that PET-avid thyroid nodules have a high risk of malignancy and should undergo FNA. Nodules detected by CT, MRI, and sestamibi, however, should first undergo a dedicated US examination. Nodules less than 1 cm in maximum diameter by US are usually not biopsied unless they have sonographically suspicious features (e.g., microcalcifications).^{14,17}

ASPIRATION TECHNIQUE AND SLIDE PREPARATION

The aspiration can be guided either by palpation or US. The benefits of palpation guidance include its reduced cost and logistical efficiency. US guidance permits the operator to be certain that the nodule of interest is aspirated (Fig. 9.1), reduces the number of unsatisfactory FNAs, and improves accuracy.^{14,18-20} For these reasons, US guidance is preferred for nonpalpable nodules, nodules that have a significant cystic component (>25%), and nodules that were previously aspirated and yielded an unsatisfactory sample.¹⁴

The aspiration technique is essentially the same whether palpation or US is used for guidance. A fine (25- or 27-gauge) needle is ideal for most thyroid nodules because the thyroid gland is vascular.²¹ Depending on the circumstances and operator preference, the aspiration can be performed with or without suction applied by a syringe. Local anesthesia by subcutaneous lidocaine injection is commonly used but is optional.²¹ The skin is wiped clean with an alcohol swab. If US guidance is used, the needle should not pass through gel, which obscures cytomorphology.²¹ The dwell time of each pass should be short, 2 to 5 seconds total in most cases, with rapid back-and-forth oscillations of the needle (3 per second) within the nodule.²¹ If suction is used, it should be released before the needle is withdrawn from the nodule. The number of passes needed to ensure adequacy varies. If a cytotechnologist or pathologist is called to evaluate the specimen for adequacy, one or two passes may be sufficient. But on-site evaluation is time consuming,²²

and many aspirations that are performed in outpatient settings are too far removed from a laboratory for such evaluation. Most practitioners recommend between two and five passes for each nodule aspirated.^{13, 21, 22} Even minor complications like a transient hematoma are uncommon; site infections are almost never seen. Post-FNA infarction of the nodule occurs to some degree in up to 10% of cases.²³ Other histologic alterations in the remaining gland include pseudoinvasion, vascular proliferation, and cytologic atypia, but these are usually easily recognized by virtue of their proximity to the linear biopsy tract.²⁴ Serious complications like needle track seeding are virtually nonexistent.

Slides are prepared by expelling and smearing the cells on a slide. Alternatively, or as an adjunct to smears, the needle is rinsed and the resulting cell suspension used for cytocentrifuge, thinlayer, or cell block preparations. The advantages of thinlayer (liquid-based) preparations over smears include reduced blood; ease in preparation of consistently well-fixed slides, particularly when the FNA is not performed by a pathologist; and a concentrated specimen that requires less screening time. When liquid-based preparation is used, one thinlayer slide is usually sufficient.²⁵ Architectural features (macrofollicles and microfollicles) are retained,²⁶ and adequacy rates and accuracy are similar for smears and thinlayer slides.²⁷⁻³¹ Some adjustment to minor morphologic alterations with liquid-based preparations is needed. For example, chronic lymphocytic thyroiditis is more subtle on thinlayer preparations because the lymphoid cells are intermingled with contaminating blood leukocytes.²⁸

Thinlayer and cytocentrifuge preparations are stained with the Papanicolaou stain. Smears can be alcohol fixed

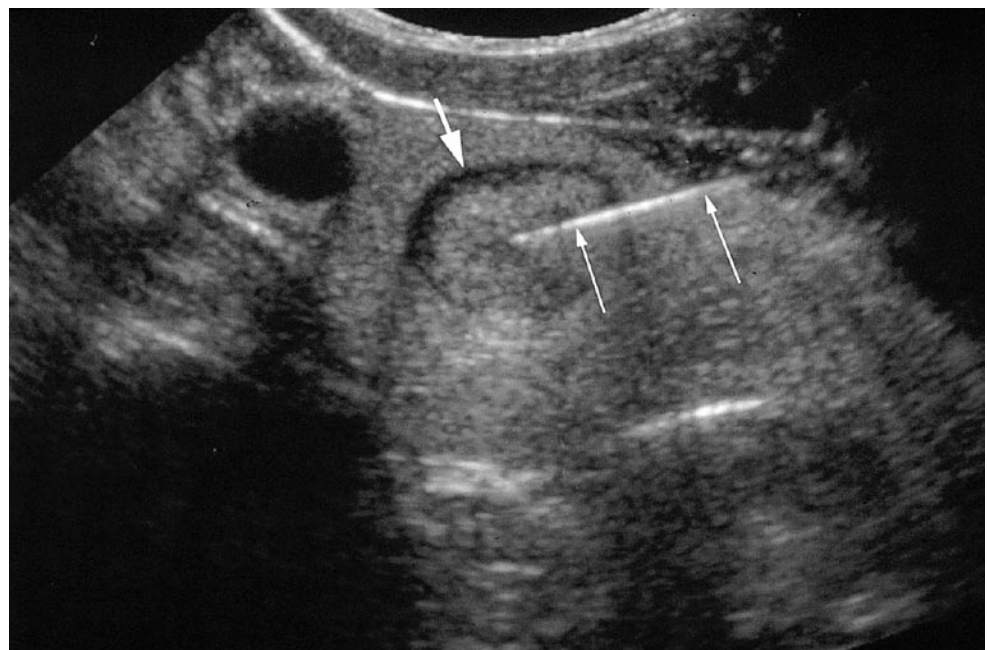


Figure 9.1 Transverse sonogram of a thyroid nodule. The tip of a needle (*thin arrows*) is in the middle of a nodule (*large arrow*). (Courtesy of Dr. Carol Benson, Brigham and Women's Hospital, Boston.)

and Papanicolaou stained or air-dried and stained with a Romanowsky-type stain. Although most cytologists prefer one over the other, it is helpful to be familiar with both stains. Nuclear features, such as inclusions, grooves, and especially chromatin texture, are better appreciated with the Papanicolaou stain. The Romanowsky-type stains are especially useful for the evaluation of extracellular material, particularly colloid and amyloid, and for cytoplasmic detail such as granules.

TERMINOLOGY FOR REPORTING RESULTS

For clarity of communication, it is advisable to begin the thyroid FNA interpretation with a general diagnostic category (e.g., benign, suspicious, malignant, etc.). A reporting system inspired by discussions at the National Cancer Institute (NCI) Thyroid Fine Needle Aspiration State of the Science Conference in 2007 is shown in [Table 9.1](#). Each of the categories has an implied cancer risk and can be linked to evidence-based clinical management algorithms: Suspicious and malignant nodules are likely to be resected, whereas patients with a benign result are instructed to return for a followup examination at an appropriate interval.

Insufficient for Diagnosis (IFD) applies to specimens that are unsatisfactory as a result of obscuring blood, overly thick smears, air-drying of alcohol-fixed smears, or an inadequate number of follicular cells. For a thyroid FNA specimen to be satisfactory for evaluation (and benign), it is customary to require at least six groups of benign follicular cells, each group composed of at least 10 cells.^{32,33} Larger groups may be split up and counted as several smaller groups of 10 cells each.³⁴ The minimum size requirement for the groups allows one to determine

(by the evenness of the nuclear spacing) whether or not they represent fragments of macrofollicles (rather than the more worrisome microfollicles). There are several exceptions to this adequacy requirement. Any specimen that contains abundant colloid is considered adequate (and benign), even if six groups of follicular cells are not identified; a sparsely cellular specimen with abundant colloid is, by implication, a predominantly macrofollicular nodule and therefore almost certainly benign. Also, whenever a specific diagnosis (e.g., Hashimoto thyroiditis) can be rendered, and whenever there is any atypia, the specimen is, by definition, adequate for evaluation. IFD results occur in 2% to 20% of cases.^{11,35,36}

Specimens that consist of cyst contents (macrophages) only ([Fig. 9.2](#)) are problematic. Many laboratories have traditionally considered a macrophages-only sample unsatisfactory because one cannot exclude a cystic papillary carcinoma. In such laboratories, macrophages-only constitute the great majority of insufficient (or nondiagnostic) cases, with nondiagnostic rates that range from 15% to 30%.^{12,36-38} Other laboratories have considered the risk of a false-negative result negligible and have reported macrophages-only as benign.^{11,37} At the 2007 Bethesda Conference, it was decided that cyst fluid cases should be considered insufficient, but that they merit a separate subcategory called “Cyst Fluid Only” (CFO). The significance (and clinical value) of an insufficient: CFO result depends on sonographic correlation. If the nodule is almost entirely cystic, with no worrisome sonographic features, an endocrinologist might proceed as if this were a benign result. On the other hand, it might require resampling if the sonographic features are worrisome and the endocrinologist is not convinced that the sample is representative. In a study that analyzed them separately from other insufficient specimens, the risk of malignancy for a CFO sample was 4%.³⁶

TABLE 9.1 BETHESDA SYSTEM FOR REPORTING THYROID FINE-NEEDLE ASPIRATION INTERPRETATIONS

Diagnostic Category	Risk of Malignancy (%)	Usual Management*
Insufficient for diagnosis	1–4	Repeat fine-needle aspiration with ultrasound guidance
Benign	<1%	Follow clinically
Atypical cells of undetermined significance	≈5–10% [†]	Repeat fine-needle aspiration
Suspicious for a follicular neoplasm	15–30	Lobectomy
Suspicious for a Hurthle cell neoplasm	15–45	Lobectomy
Suspicious for malignancy	60–75	Lobectomy or thyroidectomy
• Suspicious for papillary carcinoma • Suspicious for medullary carcinoma • Suspicious for lymphoma • Suspicious for metastatic tumor • Other		
Malignant	97–99	Thyroidectomy

*Actual management may depend on other factors besides the fine-needle aspiration result.

[†]Estimate based on resections performed after “repeated atypicals.”^{11,12}

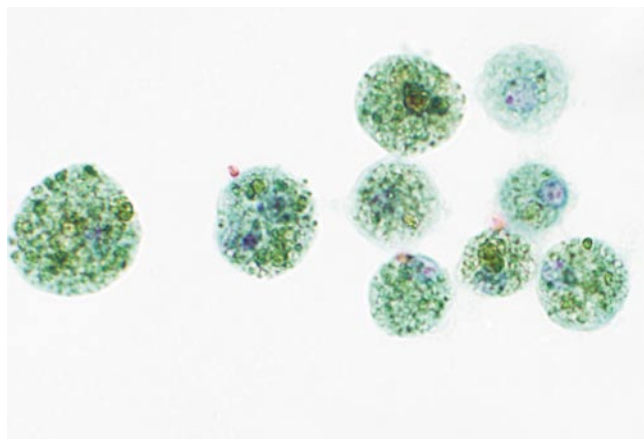


Figure 9.2 Macrophages. These cells have abundant cytoplasm that may be filled with hemosiderin or red blood cell fragments. They are a nonspecific finding, seen in both benign and malignant thyroid nodules (Papanicolaou stain).

The risk of malignancy for IFD (not including CFO) is 1% to 4%.^{11,35,36}

A repeat aspiration with US guidance is recommended for cases that are IFD and is diagnostic in 50% to 88% of cases,^{12,32,36,37,39,40} but some nodules remain persistently non-diagnostic. Because about 10% of persistently IFD nodules are malignant,³⁹ excision is considered in most cases.

A **benign** result is obtained in about 70% of thyroid FNAs. The most common benign specimen is the benign follicular nodule. The false-negative rate of a benign interpretation is quite low (<1%), but patients are nevertheless followed with repeat assessment by palpation or ultrasound at 6- to 18-month intervals.⁴¹ If the nodule shows significant growth or suspicious sonographic changes, a repeat FNA is considered.

About 10% to 15% of cases are classified as **suspicious**.^{11,12,38} These fall into three distinct subcategories: suspicious for a follicular neoplasm; suspicious for a Hürthle cell neoplasm; and suspicious for malignancy (usually papillary carcinoma).³⁸ Although virtually all cases classified as suspicious are referred for surgery, the predictive value for malignancy is different for these subcategories (see Table 9.1).

Approximately 3% to 7% of thyroid FNAs are interpreted as **malignant**, most of them as papillary carcinoma.^{11,12,37,38}

ACCURACY

The accuracy of a benign thyroid FNA result is difficult to establish because most patients with a benign result do not have surgery. Those who do undergo surgery represent a highly selected population of patients who have worrisome symptoms, nodules with substantial growth, or nodules greater than 4 cm. Nevertheless, data

indicate that a benign FNA result is highly reliable. In a long-term followup study of 439 patients with benign cytology at the Mayo Clinic, only 3 patients proved to have a malignancy, for a false-negative rate of 0.7%, and none of the patients died of their disease.³³ More recent long-term assessment data confirm a false-negative rate below 1%.¹² The most common cause of a false-negative is papillary carcinoma, followed by follicular carcinoma.^{12,33,37,42} Errors are a result in equal part of sampling and interpretation.⁴²

A malignant interpretation is likewise highly reliable; experienced practitioners have false-positive rates of 1% to 3%.^{12,38,43} although higher rates have been reported.³⁷ The most common benign tumors to cause a false-positive result are follicular adenoma (including the follicular adenoma with papillary hyperplasia) and hyalinizing trabecular tumor.^{12,37,43,44}

EVALUATION OF THE SPECIMEN

Evaluation of the specimen begins with a review of the slide(s) under scanning magnification, which quickly provides a wealth of useful information. Most benign follicular nodules are sparsely cellular, consisting predominantly of colloid. *Colloid* can be thin and translucent (“watery”); thick and opaque, with sharp outlines; or extremely thick and sticky (“bubble gum” colloid). Smears that have a high ratio of colloid to follicular cells generally indicate a benign thyroid nodule. The benign nature can be confirmed by documenting a predominance of intact macrofollicles and macrofollicle fragments (flat sheets comprised of evenly spaced follicular cells).

Neoplasms, on the other hand, are usually highly cellular specimens notable for significant *architectural atypia*, with cell crowding and overlap, and the formation of abnormal arrangements like microfollicles, trabeculae, or papillae. Microfollicles are small circles of crowded follicular cells. A predominantly microfollicular pattern is suspicious for a follicular neoplasm or a follicular variant of papillary carcinoma. Papillae—abnormal cells surrounding a fibrovascular core, often with a branching pattern—are highly characteristic of papillary carcinoma.

Examination of the slide under high magnification is important, particularly for the *nuclear changes* of papillary carcinoma, which at times are subtle and incompletely displayed.

Hürthle cells (also called oncocytes or oxyphilic cells) are metaplastic follicular cells characterized by abundant mitochondria. Why follicular cells transform into Hürthle cells is poorly understood, but they have a striking appearance on cytologic preparations: polygonal, with abundant cytoplasm that is finely granular and green or orange with the Papanicolaou stain, and

smooth and pale purple with Romanowsky-type stains. Nuclei are enlarged and sometimes pale, and nucleoli can be inconspicuous or prominent.

A predominantly *isolated cell pattern* is a nonspecific finding but is almost never seen in benign follicular nodules or papillary carcinoma. Instead, it is common in lymphocytic thyroiditis, medullary carcinoma, Hürthle cell neoplasms, poorly differentiated carcinoma, undifferentiated (anaplastic) carcinoma, and lymphoma.

Too much emphasis should not be placed on a single cytologic finding. Many features that are highly characteristic of some neoplasms can be seen sporadically in other conditions. Intranuclear pseudoinclusions, nuclear grooves, and even psammoma bodies—characteristic features of papillary carcinoma—are occasionally encountered in other conditions. Some features are entirely non-specific: *Multinucleated giant cells* are seen in subacute thyroiditis, other granulomatous diseases, benign follicular nodules with cystic degeneration, papillary carcinoma, and undifferentiated (anaplastic) carcinoma.

Rarely, a thyroid FNA is contaminated by nonthyroid cells. Accidental penetration of the larynx or trachea, virtually always announced by a cough reflex, occurs in less than 1% of thyroid FNAs,⁴⁵ and cytologic samples contain *ciliated columnar cells*.

BENIGN CONDITIONS

The most commonly encountered benign thyroid nodules are follicular cell proliferations—multinodular goiter and follicular adenoma—that account for about 70% of thyroid FNAs. Less commonly, benign nodules or pseudonodules are encountered in patients with inflammatory diseases like Hashimoto thyroiditis and subacute thyroiditis. Some benign conditions (e.g., black thyroid and radiation changes) do not cause nodules, but they produce benign cellular alterations that might be confused with malignancy.

Benign Follicular Nodules

Benign follicular nodules are encountered in patients with multinodular goiter and follicular adenoma. *Multinodular goiter (MNG)* is a common thyroid disorder characterized by an enlarged thyroid gland (goiter) with multiple areas of nodularity. Worldwide, it is the most common endocrine abnormality, affecting over 500 million people.⁴⁶ Its prevalence varies depending on regional iodine intake: Lower dietary iodine correlates with an increased prevalence of MNG. In the United States, despite iodine supplementation, the prevalence of MNG is roughly 4% to 7%.

The pathogenesis of MNG is best understood in cases associated with iodine deficiency. Because iodine is required for thyroid hormone synthesis, a deficiency

of iodine results in decreased thyroid hormone production and a compensatory elevation in serum TSH levels. Chronically elevated TSH levels stimulate a diffuse follicular cell hyperplasia. Over time, somatic mutations within the follicular cells (possibly as a result of hydrogen peroxide [H_2O_2] production and free radical generation that occurs with thyroid hormone synthesis) confer a survival advantage over selected clones, which results in nodule formation.⁴⁷ The mechanism of follicular cell hyperplasia in iodine-sufficient regions is less well understood, but it is likely related to other stimuli (e.g., smoking, radiation, drugs, naturally occurring goitrogens) superimposed on a genetic susceptibility.⁴⁷

MNG is 5 to 15 times more common in women than in men, and prevalence increases with age. Patients are usually asymptomatic and euthyroid, but 5% to 10% progress to hyperthyroidism (toxic MNG).⁴⁶ Most cases are discovered incidentally by palpation or imaging studies. MNG varies in severity, from the minimally enlarged, asymptomatic gland with only one or two nodules, to the extremely enlarged nodular gland that extends from the neck into the mediastinum. Patients with large goiters can have compressive symptoms (e.g., shortness of breath, cough, dysphagia), and large goiters can be disfiguring. The growth of nodules is unpredictable and highly variable, but, on average, MNG nodules grow by about 4.5% per year.¹⁶

The treatment of MNG is varied and individualized. Choices include thyroid surgery,¹³¹I (radioactive iodine) therapy, and TSH suppression with exogenous thyroxine (T_4) administration. Surgery is generally recommended for younger patients and those with a large goiter.¹⁶

Histologically, the nodules of MNG (commonly referred to as *adenomatous* or *adenomatoid nodules*) are usually not encapsulated. The follicles within the nodules vary in size, but most are larger than normal follicles (macrofollicles) and filled with colloid. Some nodules are comprised of such enormous macrofollicles that they are virtually all colloid (*colloid nodules*). Less commonly, a nodule may be quite cellular, comprised of smaller follicles that contain little colloid. The rapid growth of some nodules leads to hemorrhage, scarring, cystic degeneration, and dystrophic calcification. *Uninodular* MNG is a controversial entity, but some histopathologists acknowledge its existence.

Unlike MNG, a *follicular adenoma* (FA) is usually a solitary nodule that measures 1 to 3 cm in diameter, but it can be much larger.⁴⁸ The histologic distinction between a hyperplastic (adenomatous) nodule in MNG and an FA is somewhat arbitrary. In general, FA is a solitary nodule with a well-defined fibrous capsule, and the follicular cells within the nodule are morphologically distinct from those in the surrounding gland.⁴⁸ Importantly, there is no evidence of capsular or vascular invasion. FAs have a wide variety of predominant

histologic patterns: *macrofollicular* (large follicles distended by colloid); *microfollicular* (follicles smaller than normal); and *trabecular* (follicular cells are arranged in crowded ribbons).⁴⁸ Uncommon variants include FA with papillary hyperplasia (mostly in children and adolescents), signet ring cell FA, mucinous FA, lipoadenoma, clear cell FA, and FA with bizarre nuclei. Only the predominantly macrofollicular FAs are interpreted as benign follicular nodules by FNA. The others are likely to be interpreted as suspicious because of their unusual architectural patterns.

MNG and FA are essentially indistinguishable by FNA, but this is of no clinical consequence. Patients with a benign result are instructed to return for a followup examination at 6- to 18-month intervals for at least 3 to 5 years to ensure that the nodule is behaving in a benign fashion.⁴¹

CYTOMORPHOLOGY OF BENIGN FOLLICULAR NODULES:

- predominantly macrofollicles
 - fragmented (flat sheets)
 - intact spheres
- low to moderate cellularity
- cohesive cells
- uniform, evenly spaced follicular cells
- coarse chromatin
- colloid (abundant and often watery but not always visible)
- Hürthle cells (usually a minor component)
- macrophages and cyst lining cells (in cases of cystic degeneration)

A benign follicular nodule is one that has a predominantly macrofollicular architecture. During the FNA, most large macrofollicles break into fragments, and therefore intact macrofollicle spheres are relatively uncommon (Fig. 9.3A). Instead, one commonly sees flat sheets that represent fragments of macrofollicles (Fig. 9.3B). A macrofollicle fragment is defined by its flatness and the even spacing of follicular cell nuclei. (If there is conspicuous cell crowding and overlapping, the sheet is not a macrofollicle fragment.) Although the term *macrofollicle fragment* implies large size, and many are indeed large, some macrofollicle fragments are small (10 or even fewer cells). A macrofollicular pattern is defined by architecture and not size. Evenly spaced follicular cells (not crowded or overlapped) constitute a macrofollicle fragment, no matter the size of the cell sheet.

The macrofollicle fragment is made up of medium-sized cells, each with a round nucleus that has a coarsely granular chromatin texture, an inconspicuous nucleolus, and scant or moderate amounts of cytoplasm

(Figs. 9.4A, B). Cytologic atypia is generally absent, but some nuclear size variation can be seen, and in some cases the nucleoli are moderate in size. Some follicular cells contain hemosiderin pigment, which is golden-brown with the Papanicolaou stain and blue with Romanowsky-type stains.⁴⁹ Occasionally, follicular cells are arranged in microfollicles or crowded groups, but these represent a minority of the follicular cells.

Colloid is usually abundant and can take the appearance of dense blobs with a hyaline texture, hard edges, and cracking artifact (Fig. 9.5). These thick colloid chunks have a similar appearance on smears and liquid-based preparations. More often, colloid is mostly thin and watery, covering large areas of the smear as a translucent film with bubbles, folds, cracks, and a circular “chicken wire” artifact (Fig. 9.6A). On liquid-based preparations, this watery colloid has a characteristic “tissue paper” appearance (Fig. 9.6B). Colloid stains pale pink, pale green, or orange with the Papanicolaou stain, and magenta with Romanowsky-type stains.

Because most of the volume of a benign follicular nodule is occupied by colloid and not follicular cells, cytologic preparations from them tend to be sparsely cellular. But exceptions occur, and a minority is rather cellular, with numerous macrofollicle fragments.

Scattered Hürthle cells are seen in up to 50% of cases of MNG (Fig. 9.7).⁴⁹ Macrophages with foamy or hemosiderin-laden cytoplasm, including multinucleated giant cells, are often present and are a nonspecific finding indicating cystic degeneration (see Fig. 9.2).⁴⁹ A minor population of elongated, large “cyst lining cells” with pale and grooved nuclei are sometimes present. Because of their streaming (“tissue culture”) arrangement, they resemble reparative epithelial changes and likely represent altered follicular cells lining areas of cystic degeneration (Fig. 9.8).⁵⁰

DIFFERENTIAL DIAGNOSIS OF BENIGN FOLLICULAR NODULES:

- suspicious follicular nodule
- suspicious Hürthle cell nodule
- papillary carcinoma

The diagnosis of a benign follicular nodule can be made confidently when there is predominantly an orderly macrofollicular architecture. Follicular carcinomas are virtually never comprised predominantly of orderly macrofollicles, but instead have a preponderance of microfollicles or trabeculae (cords) of crowded, overlapping cells. Such cases are diagnosed as suspicious.

A minor population of crowded follicular cells or microfollicles is often present in benign follicular nodules and does not alter the benign interpretation. If

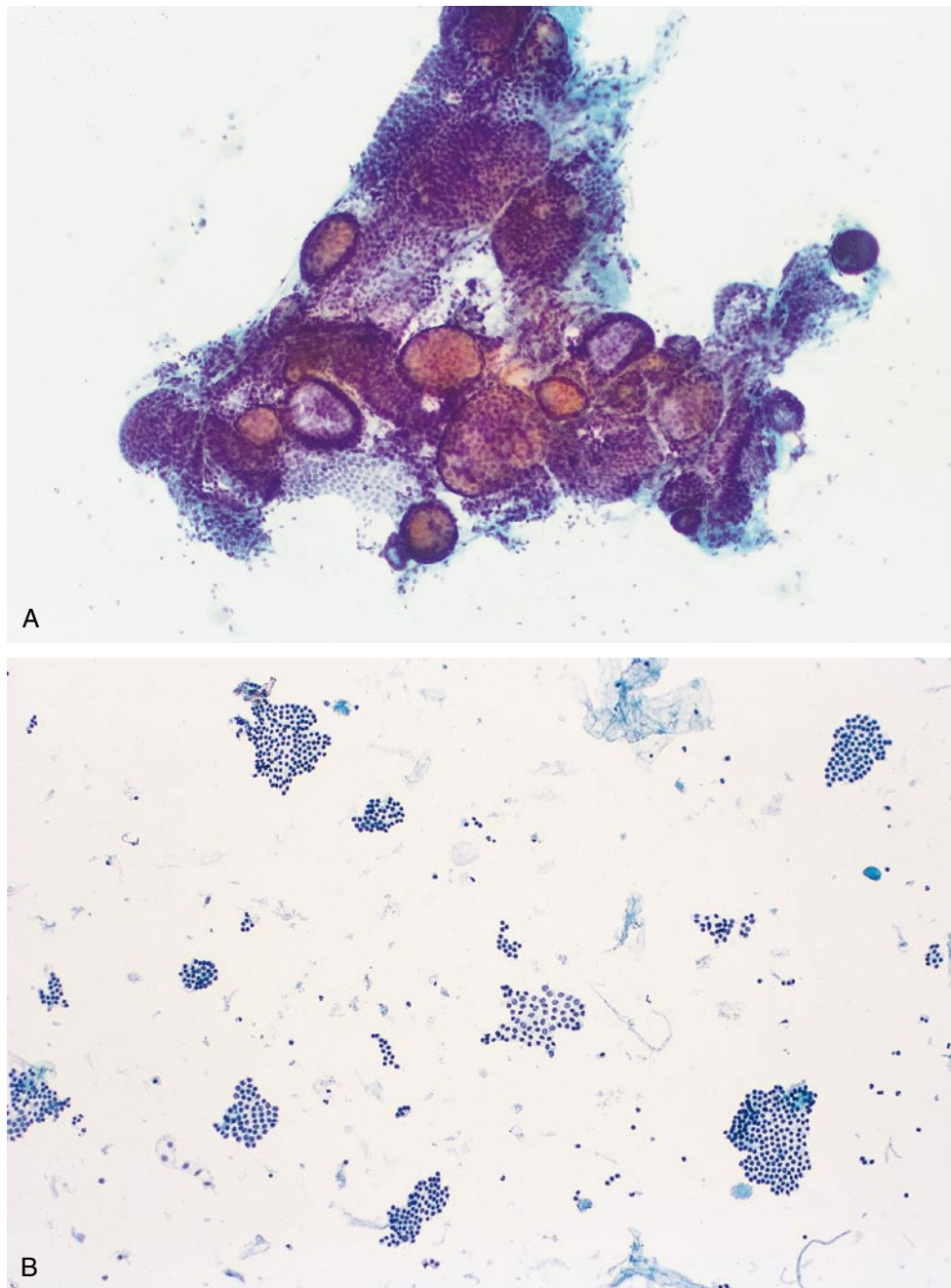


Figure 9.3 Benign follicular nodule. A, The FNA contains small tissue fragments containing intact macrofollicles (Papanicolaou stain). B, Macrofollicles often break into fragments and appear as sheets of various sizes. Although colloid is scant in this case, a predominantly macrofollicular architecture can be inferred from these fragments (Papanicolaou stain).

macrofollicles (intact or fragmented) outnumber the crowded, microfollicular or trabecular groups, the findings are consistent with a benign nodule. Follicular cells trapped in fibrin clots are often disrupted and architecturally distorted, conferring a crowded appearance that might lead to an erroneous suspicious interpretation. An interpretation should not be rendered on clotted follicular cells.

It is important to note that not all MNGs and FAs are predominantly macrofollicular. An occasional nodule in MNG and an occasional FA is comprised predominantly of microfollicles or trabeculae and is inevitably interpreted as suspicious by FNA. This is a well-known limitation of thyroid FNA. Unfortunately, there is no reliable ancillary test to assist in reducing the number of false-suspicious FNAs. Morphometry and image

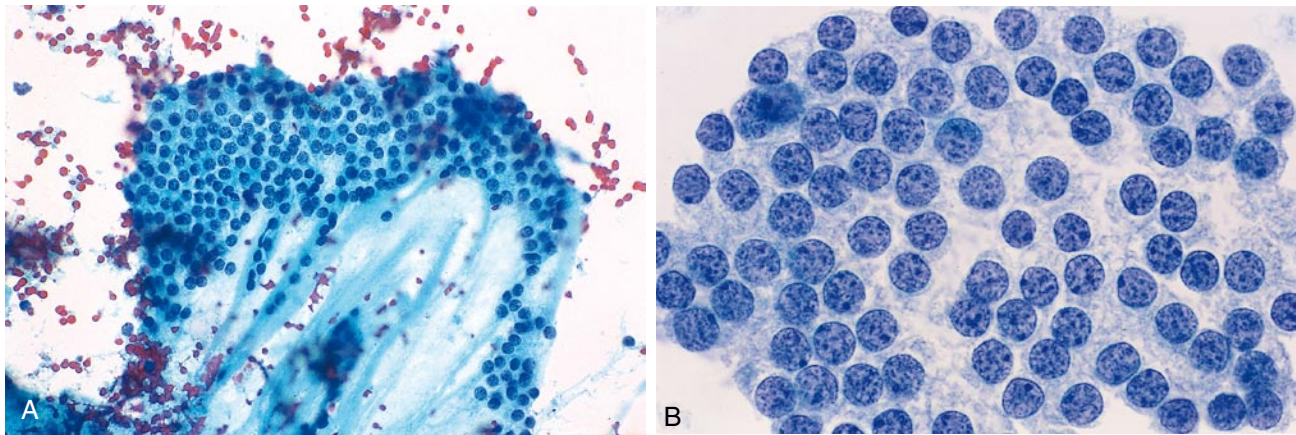


Figure 9.4 Benign follicular nodule. A, The follicular cells comprising this unfolded macrofollicle are evenly spaced. Pale green-blue colloid has escaped from the follicle (Papanicolaou stain). B, Normal follicular cells have coarsely granular chromatin, and cytoplasm can be scant or moderately abundant (Papanicolaou stain).



Figure 9.5 Colloid (benign follicular nodule). Colloid can congeal into opaque, irregularly shaped hyaline chunks with hard edges that are clearly visible on smears and liquid-based preparations (Papanicolaou stain).

analysis have not demonstrated sufficient reliability for classifying individual cases.⁵¹⁻⁵⁴ Although some separation between MNG, FA, and carcinoma is possible,⁵⁵ these methods are not sufficiently accurate to be useful for clinical diagnosis.⁵⁶ Neither is DNA quantitation by flow cytometry useful because 25% of FAs are aneuploid.⁵⁷⁻⁶⁰ Immunohistochemistry for MIB-1 is of limited value because there is significant overlap in the proliferative activity between benign and malignant follicular nodules.⁶¹ Early reports on the application of immunohistochemistry for galectin-3 appeared promising,⁶² but subsequent studies have not been able to demonstrate reliable results for FNA. An understanding of the underlying genetics of these diseases may eventually provide the key to more accurate distinction, and encouraging inroads have already been made. The translocation t(2;3)(q13;p25), which results in a *PAX8-PPAR γ* gene fusion, is

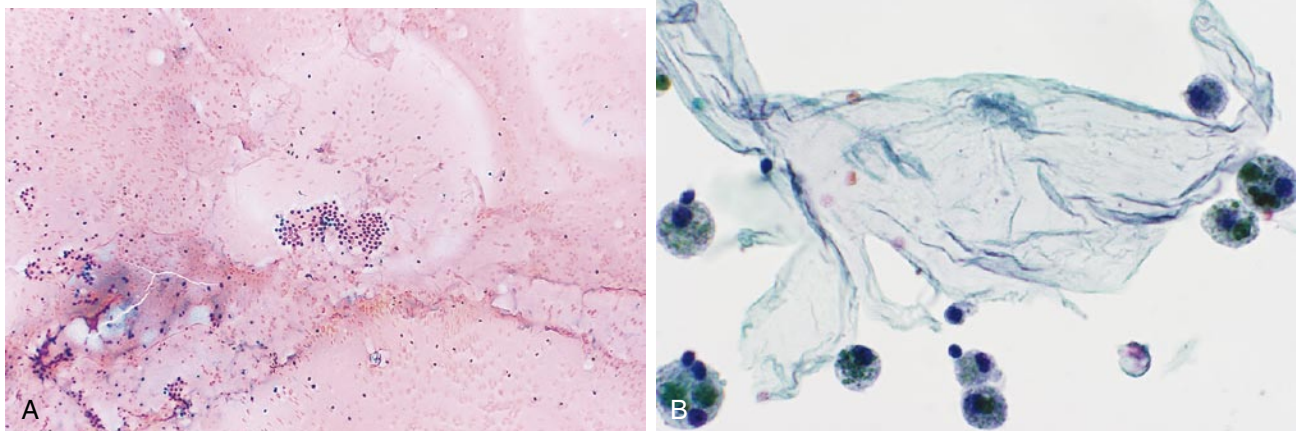


Figure 9.6 Colloid (benign follicular nodule). A, On smears, watery colloid often covers the entire slide as a thin, translucent film (pink in this image) and is often admixed with blood (Papanicolaou stain). B, On liquid-based preparations, watery colloid has a transparent, tissue-paper appearance with numerous linear folds (Papanicolaou stain).

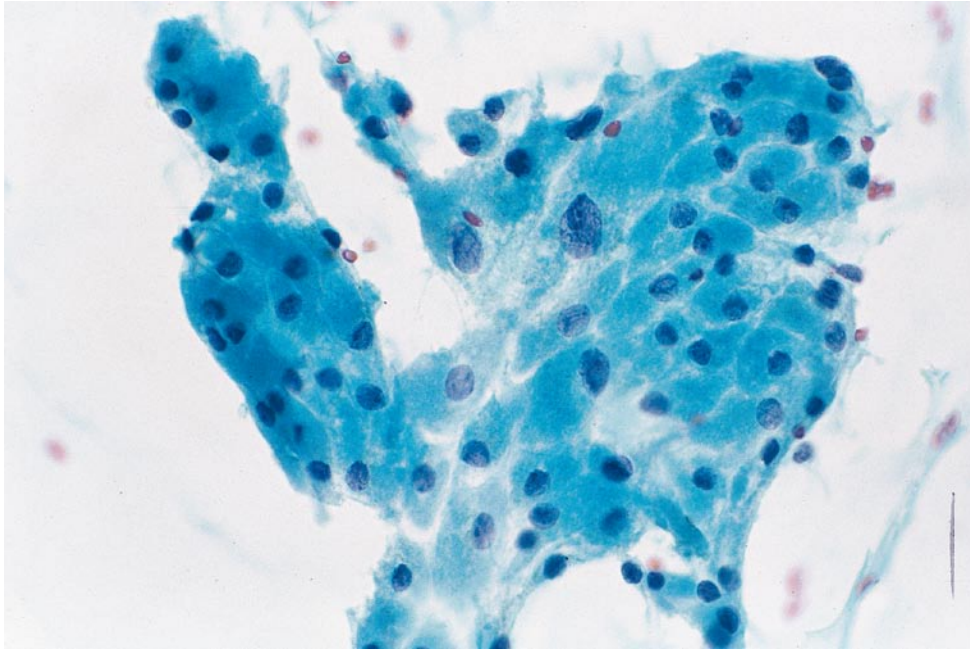


Figure 9.7 Focal Hürthle cell metaplasia, multinodular goiter (MNG). Hürthle cells are a focal finding in some cases of MNG. The cells are large and polygonal and have abundant, finely granular cytoplasm. Variation in nuclear size, as seen here, is common in non-neoplastic Hürthle cell proliferations and does not signify neoplasia (Papanicolaou stain).

a relatively specific marker of follicular carcinoma that is virtually never present in FA, MNG, or papillary carcinoma, but it has insufficient sensitivity (26% by fluorescence in situ hybridization [FISH]) for routine application to thyroid FNA.⁶³

Focal cytologic atypia is present in some benign nodules and may present diagnostic difficulties. Hürthle cell (oncocytic) metaplasia is common in MNG, and the cells sometimes show marked nuclear atypia. Such

changes raise the possibility of a Hürthle cell neoplasm. If Hürthle cells are admixed with ordinary follicular cells arranged in macrofollicles, particularly if there is abundant colloid, it is appropriate to interpret the findings as benign. By contrast, Hürthle cell neoplasms yield an exclusive population of oncocytic cells. A suspicious interpretation should be rendered only when the sample is comprised exclusively of Hürthle cells, particularly when many isolated cells are noted.

The large flat sheets of fragmented macrofollicles are reminiscent of the flat sheets often seen in papillary carcinoma. The distinction between a benign nodule and papillary carcinoma depends primarily on careful examination of nuclear features. The distinction can be difficult in some cases, particularly with the macrofollicular variant of papillary carcinoma, in which the nuclear changes are often subtle and focal.⁶⁴ Other than differences in the nuclei, the malignant cells of papillary carcinoma are arranged more haphazardly, with more crowding, than the follicular cells of benign nodules. In rare cases, macrophages in MNG can aggregate, and their large pale nuclei might be mistaken for papillary carcinoma.⁶⁵

The large, pale nuclei of cyst lining cells bear a resemblance to the cells of papillary carcinoma. When the nuclear changes are mild and the great majority of the sample looks benign, the benign, reactive nature of these cells is easily recognized. In some cases, the nuclear changes can be marked, and papillary carcinoma cannot be excluded. Depending on the degree of atypia, such cases may need to be interpreted as atypical cells of undetermined significance or even suspicious for papillary carcinoma.⁵⁰

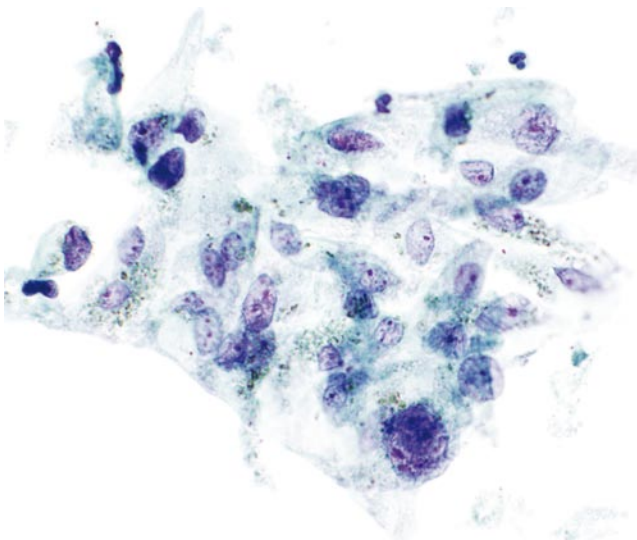


Figure 9.8 Cyst lining cells. Reactive follicular cells are often seen adjacent to areas of cystic degeneration. They typically have a flat, cohesive appearance, with enlarged, oval nuclei, some of which are pale and grooved. In some cases there is intracytoplasmic hemosiderin (Papanicolaou stain).

Chronic Lymphocytic (Hashimoto) Thyroiditis

Chronic lymphocytic (Hashimoto) thyroiditis (HT) is a common autoimmune disease in which thyroid follicles are destroyed by a marked lymphoid infiltrate. Almost 95% of HT occurs in women, with a peak incidence between ages 40 and 60. The disease results in a diffuse, painless goiter with or without nodularity. Most patients are hypothyroid. Histologic examination reveals an intense, patchy lymphoid infiltrate with germinal centers, follicular atrophy, and Hürthle-cell metaplasia. Fibrosis is prominent in a minority of cases. The diagnosis is established by correlating clinical findings with serologic test results. One or more of a variety of circulating autoantibodies are identified in almost all patients; the most common are anti-thyroglobulin and anti-thyroid peroxidase (TPO). (TPO was originally called the *microsomal antigen*.) In a patient with HT, an FNA is performed only if there is a suspicious nodule that raises the possibility of a coexisting malignancy.

CYTOMORPHOLOGY OF CHRONIC LYMPHOCYTIC (HASHIMOTO) THYROIDITIS:

- mixed population of lymphocytes
- tingible-body macrophages
- dendritic-lymphocytic aggregates
- Hürthle cells ± follicular cells
- occasional follicular or Hürthle cells with mild nuclear pallor and occasional grooves
- scant colloid

The aspirate is usually cellular, with numerous dispersed, heterogeneous lymphoid cells, including small lymphocytes, centrocytes, centroblasts, and dendritic cells (Fig. 9.9A, B). With liquid-based preparations, the dispersed lymphoid component is admixed with the white blood cells from contaminating blood. Dendritic-lymphocytic aggregates (germinal center fragments containing dendritic cells) are also present, as are tingible-body macrophages. Colloid is usually absent or scant. Normal follicular cells are infrequent or absent altogether; instead, there may be occasional clusters of Hürthle cells (Fig. 9.9C). The Hürthle cells and any residual follicular cells occasionally display focal chromatin clearing and nuclear grooves. Multinucleated giant cells can be present but are usually not as numerous as in subacute thyroiditis.

DIFFERENTIAL DIAGNOSIS OF CHRONIC LYMPHOCYTIC (HASHIMOTO) THYROIDITIS:

- reactive lymph node
- MNG with prominent Hürthle cell change
- primary thyroid lymphoma
- Hürthle cell neoplasm
- papillary carcinoma

The cytologic diagnosis of HT is usually straightforward and is confirmed clinically by serologic tests for antibodies against the aforementioned thyroid antigens. Pitfalls include aspiration of a reactive lymph node (unlikely if the FNA was US-guided) and MNG with prominent Hürthle cell changes. Prominent Hürthle cell change occurs in some cases of MNG, but a lymphoplasmacytic infiltrate is absent or sparse.

Primary thyroid lymphoma is an uncommon malignancy that must be considered in the differential diagnosis of HT. Almost all cases of thyroid lymphoma arise in patients with longstanding HT, and patients with HT have a significantly higher relative risk for developing thyroid lymphoma compared to the general population.⁶⁶ Histologically, primary thyroid lymphomas are either extranodal marginal zone B-cell lymphomas, diffuse large B-cell lymphomas, or a mixture of the two.⁴⁸ Cytologically, thyroid lymphomas are composed of a uniform population of either small or large lymphoid cells. Because lymphoma is uncommon, immunophenotyping is not performed routinely on cases of typical HT and might actually be counterproductive. Some HT specimens contain clonal B-cell populations (demonstrated by a restricted kappa-lambda light chain ratio by flow cytometry or an immunoglobulin H [IgH] gene rearrangement by the polymerase chain reaction) that do not equate to malignancy and thus can be misleading.^{67,68} For this reason, immunophenotyping or molecular genetics are recommended only if the clinical presentation (rapid growth, large nodule) or cytomorphology (monomorphous or atypical lymphoid population) suggest lymphoma.

Hyperplastic Hürthle cell nodules occur in HT and mimic a Hürthle cell tumor. The cells of a Hürthle cell tumor, however, usually have a more prominent nucleolus than those of HT, and a prominent lymphoid infiltrate is absent. In a patient with a history of HT, a nodule is more likely to represent a hyperplasia of Hürthle cells rather than a Hürthle cell neoplasm. In a minority of cases this distinction may nevertheless be hard to make, and definitive classification may depend on histologic examination.

The Hürthle cells (and any residual follicular cells) of HT occasionally display focal chromatin clearing and nuclear grooves. When mild and focal, such changes usually do not correlate with a histologic papillary carcinoma and can be disregarded on an FNA. If they are widespread and associated with other features of papillary carcinoma, however, a suspicious or malignant interpretation is warranted.

Subacute (de Quervain) Thyroiditis

Subacute (de Quervain) thyroiditis (SAT) is a rare, painful enlargement of the thyroid gland in which thyroid follicles are damaged by a chronic granulomatous inflammatory reaction, possibly resulting from a viral

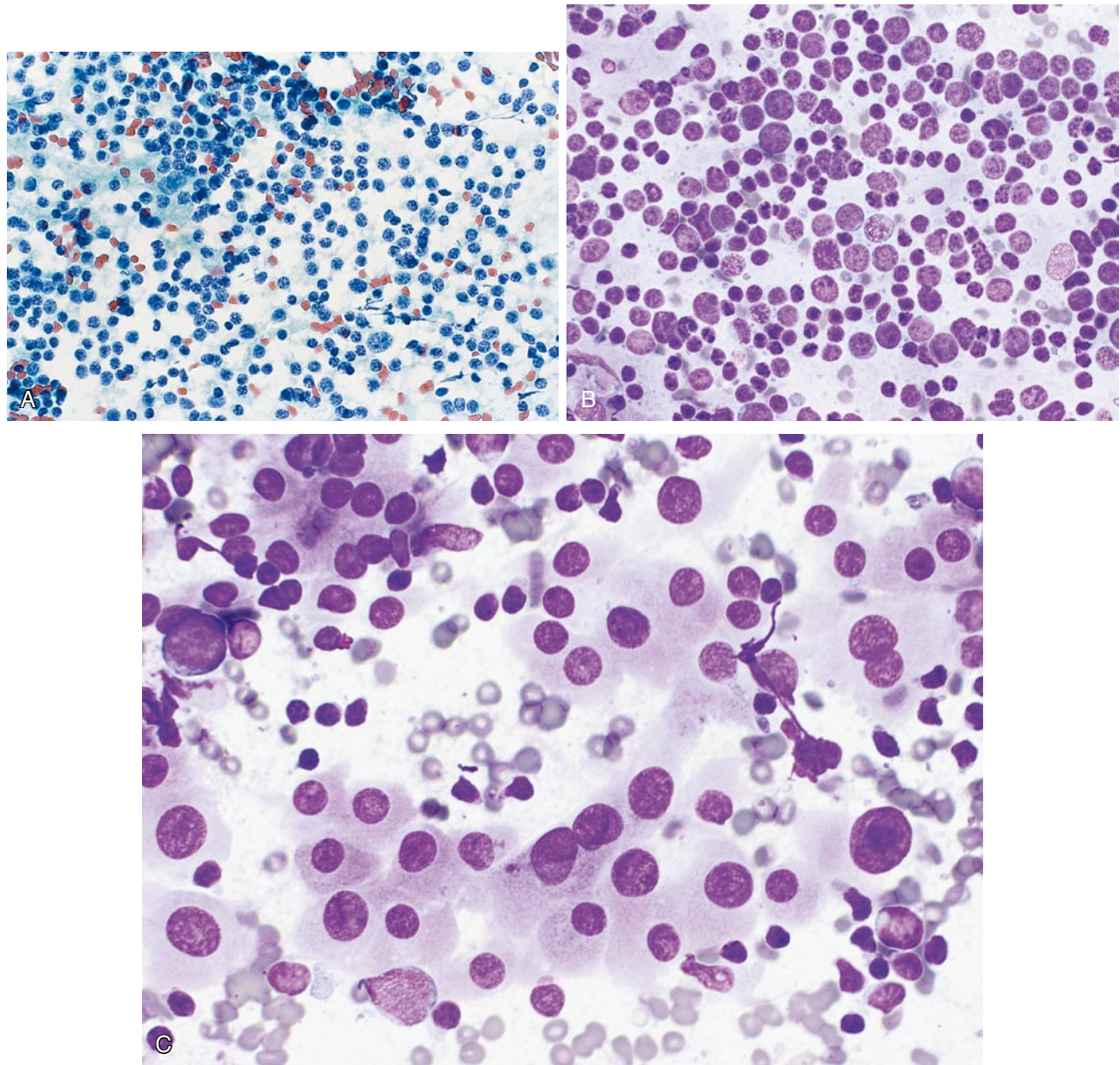


Figure 9.9 Hashimoto thyroiditis (HT). A, Lymphoid cells are the predominant feature. Most are small, mature lymphocytes (Papanicolaou stain). B, The heterogeneity of the lymphoid cells is better appreciated with air-dried preparations (Wright-Giemsa stain). C, Hürthle cells with abundant cytoplasm are usually identified in clusters in HT. Note the scattered lymphoid cells (Wright-Giemsa stain).

infection. Early, transient hypothyroidism is quite common, but permanent hypothyroidism occurs only in a minority of patients. SAT usually lasts several months and is self-limited, but it can recur in about 4% to 9% of patients.^{69,70} Patients are treated with nonsteroidal anti-inflammatory medications, and in more severe cases, with corticosteroids. In most cases, FNA plays no role in the diagnosis, which is usually established clinically. Because the gland in SAT can appear inhomogeneous, however, an FNA is sometimes performed to rule out a malignant nodule.

CYTOMORPHOLOGY OF SUBACUTE THYROIDITIS:

- multinucleated giant cells
- granulomas (rare)
- lymphocytes

Aspirates can be sparsely, moderately, or highly cellular.⁷¹ Multinucleated giant cells are usually a prominent, striking finding. They are numerous and large, with bizarre, angular shapes (Fig. 9.10A). In SAT, they tend to

have densely granular but not vacuolated cytoplasm, in contrast with the frothy cytoplasm typical of the multinucleated cells in MNG nodules with cystic degeneration.⁷² Some multinucleated cells have ingested colloid droplets.⁷² Granulomas (Fig. 9.10B) are the hallmark of the disease but are not always present. Granulomas are crowded aggregates of epithelioid histiocytes that have an oval, spindle-shaped, kidney-shaped, or curved (“boomerang”) nucleus and abundant granular or vacuolated cytoplasm. Cell membranes are poorly defined, and as a result, granulomas have a syncytium-like appearance. Granulomas, if present, are rare and need to be hunted for in most cases.⁷¹ Scant unremarkable macrofollicle fragments and colloid are usually present.⁷¹

The cytologic differential diagnosis includes other granulomatous conditions like sarcoidosis and tuberculous infection, which can be excluded clinically and with microbiologic studies. Multinucleated giant cells by themselves are not diagnostic of SAT because they are seen in a variety of benign and malignant conditions, including papillary carcinoma and undifferentiated (anaplastic) carcinoma. The pale, elongated, or folded nuclei of the epithelioid histiocytes of a granuloma might cause them to be misinterpreted as papillary carcinoma nuclei. Undifferentiated (anaplastic) carcinoma can usually be ruled out because of the absence of significant nuclear atypia.

Riedel Disease

This rare entity of unknown cause results in a hard thyroid mass that clinically mimics undifferentiated (anaplastic) carcinoma. It is characterized by a dense fibrosis that replaces the thyroid parenchyma and extends beyond the thyroid to surrounding structures. The gland may be of normal size or slightly enlarged and is often asymmetric or nodular

on clinical examination.⁷³ Because of the dense fibrosis, aspiration often results in a dry tap,⁴³ and surgical biopsy is often necessary for a diagnosis. FNA sometimes reveals fragments of fibrous tissue and plump myofibroblasts with abundant cytoplasm.⁷⁴ A mixed inflammatory infiltrate of lymphocytes, plasma cells, neutrophils, and rare eosinophils is seen. A fibrosing variant of HT is excluded by clinical correlation and the absence of germinal center fragments and Hürthle cells. Multinucleated giant cells and granulomas are absent, excluding SAT.

Amyloid Goiter

Amyloid goiter is a focal or diffuse enlargement of the thyroid gland, sometimes with alarming clinical symptoms such as rapid growth, dyspnea, dysphagia, or hoarseness. Most patients have a chronic illness predisposing them to systemic amyloidosis. The thyroid interstitium is infiltrated by masses of amyloid, some surrounded by multinucleated giant cells. The diagnosis can be established by FNA.^{75,76} Amyloid is similar to colloid, except that the amyloid in amyloid goiter contains stretched and distorted fibroblast nuclei⁷⁵ (Fig. 9.11). A conclusive diagnosis rests on identifying the characteristic apple-green dichroism with the Congo red stain. Follicular cells are scant.

Amyloid often accompanies medullary carcinoma and in some cases overshadows the cellular component (amyloid-rich medullary carcinoma).

Black Thyroid

A benign but striking dark-brown pigmentation of thyroid follicular cells occurs in patients who take antibiotics of the tetracycline group (e.g., minocycline) for long periods of time, most commonly for acne. This pigmen-

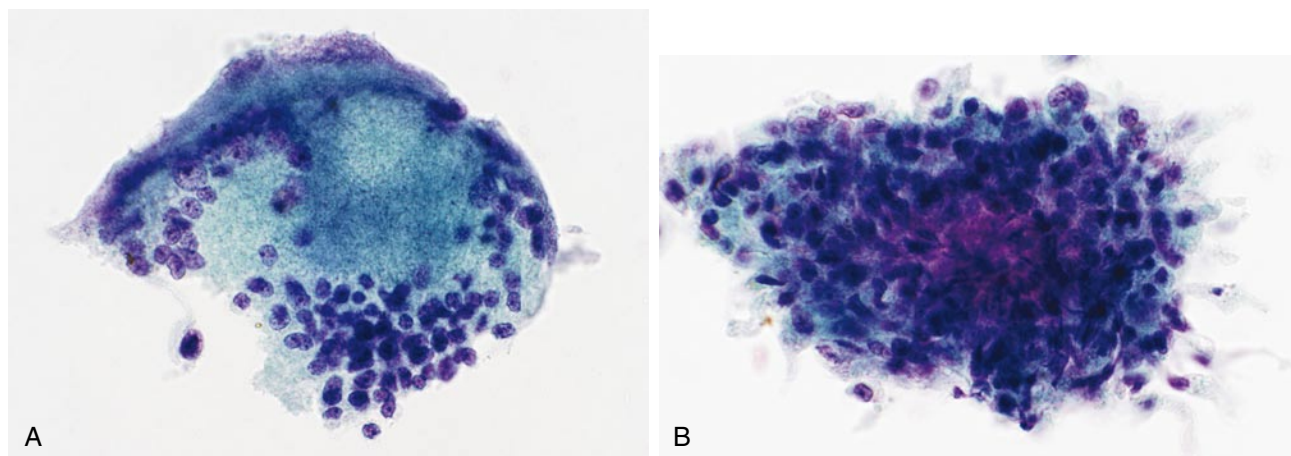


Figure 9.10 Subacute thyroiditis. A, The most conspicuous finding is an abundance of multinucleated giant cells with bizarre shapes (Papanicolaou stain). B, Granulomas are often few in number. The nuclei of epithelioid histiocytes have a variety of elongated and curved shapes. Cytoplasm is abundant, clear, and poorly demarcated (Papanicolaou stain).

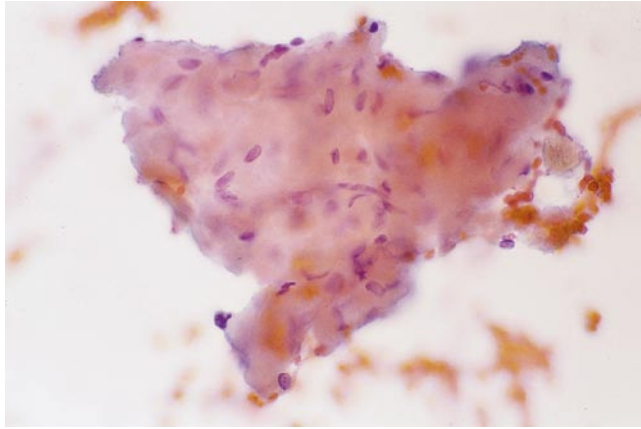


Figure 9.11 Amyloid goiter. Like colloid, amyloid forms opaque masses with irregular, sharp edges. Amyloid is deposited in the interstitium in patients with amyloid goiter, hence aspirated amyloid fragments often contain entrapped fibroblasts (Papanicolaou stain).

tation is so prominent in some cases that the gland is black on gross inspection. Cytologic preparations show abundant dark brown pigment granules within the cytoplasm of follicular cells (Fig. 9.12). The pigment is a darker brown than hemosiderin. Because the pigment stains with the Fontana stain, it is thought to represent a type of melanin. Recognizing this as a benign condition may prevent unnecessary surgery in these patients.^{77,78}

Radiation Changes

Both external radiation to the neck and systemic administration of radioactive iodine (^{131}I) can cause long-term morphologic changes in the thyroid gland. External radiation is used in low doses to treat a variety of benign conditions, and in high doses for malignancies like Hodgkin disease. Radioactive iodine is administered to treat hyperthyroidism, whether as a result of Graves disease, MNG, or a functioning thyroid carcinoma.

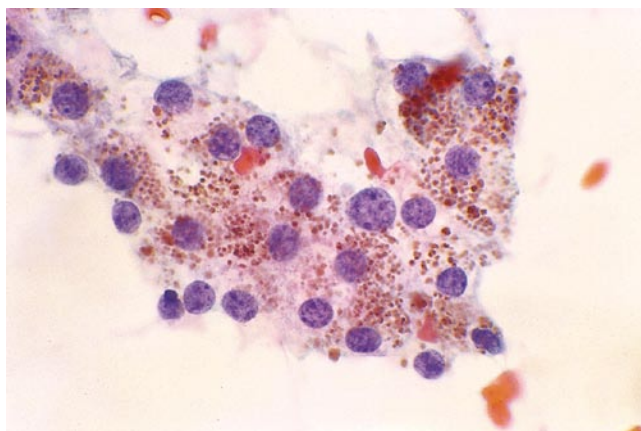


Figure 9.12 Black thyroid. Follicular cells contain abundant coarse, brown cytoplasmic granules (Papanicolaou stain).

CYTOMORPHOLOGY OF RADIATION CHANGES:

- sheets (macrofollicle fragments)
- enlarged cells
- normal nuclear-to-cytoplasmic ratio
- Hürthle cell change
- cytoplasmic vacuolization
 - marked nuclear atypia:
 - marked size variation
 - hyperchromasia
 - smudged chromatin
 - grooves, pseudoinclusions
 - naked nuclei

Follicular cells altered by radiation are arranged in sheets, and nuclei vary greatly in size, some reaching giant proportions (Fig. 9.13). Chromatin is dark, coarsely granular, and nucleoli can be prominent. Cytoplasm is abundant, suggestive of Hürthle cell change, and sometimes vacuolated.

The differential diagnosis includes follicular carcinoma, papillary carcinoma, and undifferentiated (anaplastic) carcinoma^{79,80}—important considerations given that external radiation has been associated with an increased risk of thyroid cancer. (No association between ^{131}I and cancer has been demonstrated.) Although the atypia seen with radiation is marked, there is little in the way of microfollicular architecture, a hallmark of follicular neoplasms. The isolated cell pattern of an undifferentiated (anaplastic) carcinoma is not seen. Although some of the nuclear features characteristic of papillary carcinoma are occasionally seen (grooves, pseudoinclusions), in other ways (marked hyperchromasia and nuclear size variation) the findings are not typical. Similar changes are induced by other drugs used to treat Graves disease, such as methimazole and carbimazole.⁸¹

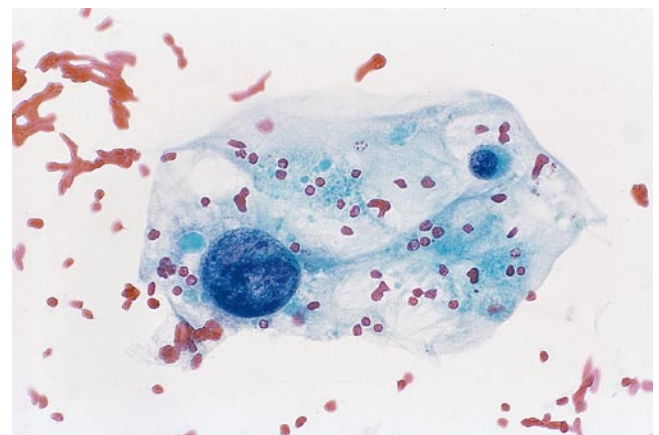


Figure 9.13 Radioactive iodine effect. Follicular cells show marked variation in cellular and nuclear size, sometimes with conspicuous cytoplasmic vacuolization (Papanicolaou stain).

ATYPICAL CELLS OF UNDETERMINED SIGNIFICANCE

Some thyroid FNAs are not easily classified into the benign, suspicious, or malignant categories. Such cases represent the “I don’t know” category and in the Bethesda System are reported as atypical cells of undetermined significance (ACUS). Examples include a sparsely cellular specimen but one that shows a predominance of microfollicles or a case with rare but atypical cells that might represent cyst lining cells but whose atypia extends beyond the usual, readily identifiable benign features.⁵⁰ Similarly, a specimen composed exclusively of Hürthle cells is usually interpreted as suspicious, but in a patient with HT it most likely represents a benign hyperplastic Hürthle cell nodule and might be downgraded to ACUS with an explanatory note.

An ACUS result is obtained in 3% to 6% of thyroid FNAs.^{11,12} The recommended management is clinical correlation and a repeat FNA at an appropriate interval.^{12, 41} In most cases, a repeat FNA results in a more definitive interpretation; only about 20% of nodules are repeatedly ACUS.¹² The risk of malignancy for an ACUS nodule is difficult to ascertain because only a minority of cases in this category have surgical follow-up. Those that are resected represent a selected population of patients with repeatedly ACUS results or patients with worrisome clinical or sonographic findings. In this selected population, 20% to 25% of patients with ACUS prove to have cancer after surgery, but this is undoubtedly an overestimate for all ACUS nodules.^{11,12} Extrapolating for all ACUS nodules, the risk of malignancy is probably closer to 5% to 10% (see Table 9.1). An effort should be made to use this category as a last resort and limit its use to fewer than 7% of all thyroid FNAs.

SUSPICIOUS FOR A FOLLICULAR NEOPLASM

The purpose of this diagnostic category is to identify a nodule that might be a follicular carcinoma and triage it for surgical excision. FNA is diagnostic of many thyroid conditions (e.g., papillary carcinoma, HT, etc.), but with regard to follicular carcinoma, it is better considered a screening test.

Follicular carcinoma (FC) is the second most common malignant thyroid cancer and comprises 10% to 15% of thyroid malignancies.⁴⁸ Together with papillary carcinoma and Hürthle cell carcinoma, FC is by definition one of the “differentiated carcinomas,” but it is biologically more aggressive than papillary carcinoma, tending to metastasize to the lung and bones and less often to regional lymph nodes. FC is commonly divided into two subtypes: a minimally invasive carcinoma with an excellent prognosis and a widely invasive malignancy with a poor prognosis.

Histologically, FC is distinguished from FA by the presence of capsular penetration, vascular invasion, or both. One cannot make a diagnosis of FC from a cytologic specimen because this tumor is defined by histologic criteria of invasion that cannot be assessed by FNA. Historically, when a specific “histologic” diagnosis of FA or FC was valiantly attempted, the results were disappointing: one third of cases diagnosed as FC turned out to be FAs, and 1 in 10 specimens called FAs by FNA proved to be carcinomas after histologic examination.⁴³ Nevertheless, FCs have distinctive neoplastic features that are recognizable and reportable as suspicious by cytology, leading to a definitive diagnostic procedure, usually lobectomy.^{38,41,82} About 15% to 30% of cases labeled suspicious for a follicular neoplasm prove to be malignant.^{11,12,42,83} The majority of cases in this suspicious category turn out to be FAs or adenomatoid nodules of MNG, both of which are more common than FC. Of those that prove to be malignant, many are FCs, but a significant proportion are follicular variants of papillary carcinoma.^{12,35,37,83}

After surgery, if histological examination reveals an adenoma (or other benign nodule), the patient requires no further therapy. If carcinoma is found, a completion thyroidectomy may be necessary because a large thyroid remnant obscures subsequent ¹³¹I uptake studies used to detect recurrent/metastatic FC. FC metastases accumulate ¹³¹I optimally only in the absence of normal thyroid tissue.⁸²

CYTOMORPHOLOGY OF SUSPICIOUS FOR A FOLLICULAR NEOPLASM:

- marked cellularity
- predominantly microfollicles or trabeculae
- enlarged, crowded follicular cells
- scant colloid

Cytologic preparations typically have high cellularity, and colloid is scant or absent. The follicular cells are arranged predominantly in microfollicular (Fig. 9.14A) or trabecular (Fig. 9.14B) arrangements. Cellular crowding and overlapping are conspicuous, and the follicular cells are usually larger than normal. Nuclear atypia or pleomorphism and mitoses are uncommon. A minor population of macrofollicles (intact spheres and fragments) can be present.

DIFFERENTIAL DIAGNOSIS OF SUSPICIOUS FOR A FOLLICULAR NEOPLASM:

- benign follicular nodule
- papillary carcinoma
- metastatic renal cell carcinoma
- parathyroid adenoma or carcinoma

Marked cellularity alone does not qualify the nodule for a suspicious interpretation.¹⁰ If the sample is cellular but mostly macrofollicular (intact spheres and

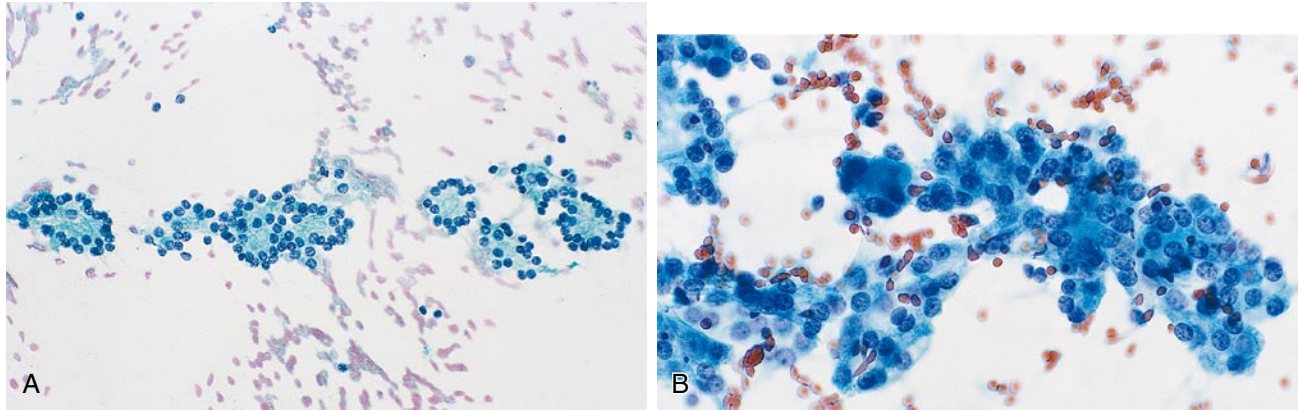


Figure 9.14 Suspicious for a follicular neoplasm. A, A neoplasm should be suspected whenever a specimen is composed predominantly of microfollicles (Papanicolaou stain). B, The cells of some follicular neoplasms are crowded haphazardly into ribbons (trabeculae; Papanicolaou stain).

flat fragments), a benign interpretation is appropriate. Benign follicular nodules often have a small population of microfollicles and crowded groups. If these comprise the minority of the follicular cells, they have little significance, and the FNA can be interpreted as benign. A suspicious interpretation is rendered when the majority of the follicular cells are arranged in abnormal architectural groupings (microfollicles, crowded trabeculae).

The differential diagnosis includes other thyroid neoplasms. If features of papillary carcinoma (nuclear grooves, pseudoinclusions, etc.) are present, the category “suspicious for a follicular neoplasm” is not appropriate. The case should instead be reported as suspicious for malignancy (papillary carcinoma) or malignant, depending on the quantity and quality of the cytomorphologic changes. Metastatic renal cell carcinoma can mimic a follicular neoplasm. A clinical history of renal cell carcinoma is helpful to alert the cytopathologist to this possibility and ideally is provided on the requisition that accompanies the FNA.¹⁴ Some renal cell carcinomas, however, are occult at the time of thyroid metastases. The rare *clear cell variant of FC* is composed of cells with abundant cytoplasm and is morphologically indistinguishable from metastatic renal cell carcinoma and some parathyroid tumors.⁸⁴ Parathyroid adenomas are virtually impossible to distinguish from a follicular neoplasm by cytomorphology alone. A clinical suspicion of a parathyroid neoplasm is invaluable for the cytopathologist because it may prompt additional evaluation with immunohistochemistry. Immunostains for thyroglobulin and thyroid transcription factor-1 (TTF-1) are positive in follicular neoplasms and negative in parathyroid adenomas, which are immunoreactive instead for parathyroid hormone.

SUSPICIOUS FOR A HÜRTHLE CELL NEOPLASM

The purpose of this diagnostic category is to identify a nodule that might be a **Hürthle cell carcinoma** (HC)

and triage it for surgical excision. As it is for FC, FNA is better considered a screening rather than a diagnostic test for HC. In the World Health Organization’s (WHO) classification, Hürthle cell adenoma (HA) and HC are considered oncocyctic variants of FA and FC, respectively.⁴⁸ Studies suggest, however, that follicular and Hürthle cell tumors have different underlying genetics.⁶³

HC accounts for 3% to 4% of thyroid cancers. In contrast with FCs, which rarely present with lymph node metastases, HCs are associated with nodal metastases in 30% of cases.⁴⁸ They tend to be larger than HAs and present in older patients. Histologically, HCs show follicular, trabecular, or solid growth patterns. The nuclei have a prominent nucleolus and there is abundant granular cytoplasm. Colloid, when present, sometimes undergoes a curious basophilic transformation with concentric calcification. These structures are difficult to distinguish from psammoma bodies. Clear cell change is prominent in some HCs. HCs and HAs are immunoreactive for thyroglobulin and TTF-1.

Distinction between HA and HC, as with their conventional follicular counterparts, rests with identifying capsular or vascular invasion. Invasion cannot be established by FNA, but a suspicion of neoplasia (based on the criteria outlined herein) can be raised that leads to a definitive diagnosis after lobectomy. The initial management is almost identical to that for patients with a cytologically suspicious follicular nodule, and unless there is clinical evidence of invasion, a lobectomy is advised. If the nodule proves to be malignant after histologic examination, a completion thyroidectomy is recommended.⁸²

Most nodules interpreted as suspicious for a Hürthle cell neoplasm prove to be neoplasms, but 10% to 25% turn out to be non-neoplastic nodules in MNG or HT.^{43,85-87} Fifteen percent to 45% of the nodules are malignant (see [Table 9.1](#)), and the remainder of the neoplasms prove to be HAs.^{42,86,87}

CYTOMORPHOLOGY OF SUSPICIOUS FOR A HÜRTHLE CELL NEOPLASM:

- pure Hürthle cell population
- usually dyshesive cells
- prominent nucleolus
- pseudopsammoma bodies

A Hürthle cell neoplasm is suspected if the aspirate is composed exclusively of Hürthle cells (Fig. 9.15). These are often isolated cells, but groups, usually crowded and syncytium-like, can be seen.^{85,88} A large nucleolus is more typical of neoplastic than hyperplastic Hürthle cells.^{85,88} Colloid can be present, but abundant lymphocytes and normal follicular cells are absent. Basophilic structures with concentric lamellae are seen in some cases and are often indistinguishable from psammoma bodies.

It has been suggested that the diagnostic criteria for this category should be narrowed to include only those cases in which the Hürthle cells show additional abnormalities, either cytologic (small cell dysplasia, large cell dysplasia) or architectural (crowding or marked dyshesion).⁸⁹ The goal of the stricter criteria is to reduce the number of patients undergoing unnecessary lobectomy.

DIFFERENTIAL DIAGNOSIS OF SUSPICIOUS FOR A HÜRTHLE CELL NEOPLASM:

- HT
- MNG
- macrophages
- papillary carcinoma
- metastatic renal cell carcinoma
- medullary carcinoma

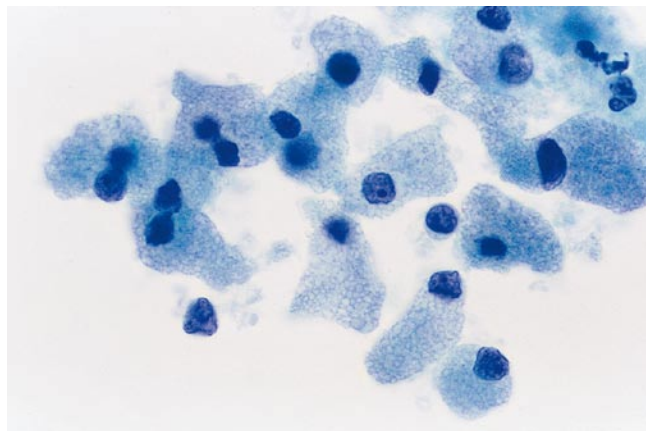


Figure 9.15 Suspicious for a Hürthle cell neoplasm. The sample is composed of numerous Hürthle cells and little else (Papanicolaou stain).

Hürthle cells are not neoplastic but merely a component of HT when they are admixed with numerous lymphoid cells (see Fig. 9.9A-C). Similarly, they are not neoplastic when admixed with macrofollicles and colloid, a heterogeneous mixture that is typical of MNG. Cellular features of the Hürthle cells themselves can help in this regard. Neoplastic Hürthle cells often and have a prominent nucleolus. In HT and MNG, the cells can be pleomorphic, with marked anisonucleosis and hyperchromasia, but macronucleoli are usually absent. Macrophages can sometimes be confused with Hürthle cells and vice versa, particularly with liquid-based preparations, in which the usually granular cytoplasm of Hürthle cells appears microvacuolated. But the pseudovacuumization of Hürthle cells in liquid-based preparations is fine and does not have the coarsely vacuolated appearance of macrophage cytoplasm. In addition, Hürthle cells generally lack hemosiderin and are more polygonal, rather than rounded like macrophages.

If an FNA consists exclusively of Hürthle cells but the nodule is relatively small (<2.5 cm) and the patient has HT, the nodule is most likely a hyperplastic nodule. In that circumstance, downgrading the interpretation to ACUS (with an explanatory note) can be considered. The same applies to a patient known to have multiple nodules, in whom the (small) nodule is likely to represent Hürthle cell transformation of an adenomatoid nodule.

The differential diagnosis includes variants of papillary carcinoma (tall cell and oncocytic), but these can be excluded by the absence of nuclear features diagnostic of papillary carcinoma. It is worth noting that the nuclei of Hürthle cells can sometimes be paler than those of normal follicular cells. This can create diagnostic difficulty, but if there are no other convincing features of papillary cancer, the cells are most likely Hürthle cells. Similar confusion with papillary carcinoma may occur because some Hürthle cell tumors have calcific structures that resemble psammoma bodies.⁴⁸ Because HAs and HCs can have clear cell features, metastatic renal cell carcinoma mimics a Hürthle cell neoplasm to perfection (Fig. 9.16). A clinical history of renal cell carcinoma can alert the cytopathologist to this possibility and should be provided on the requisition.¹⁴ Immunohistochemistry for thyroglobulin and TTF-1 are helpful because the cells of renal cell carcinoma are negative for these markers.

The differential diagnosis includes medullary carcinoma. A dispersed cell pattern is common in both, and the cells of both tumors can have a plasmacytoid appearance. A prominent nucleolus, however, is rare in medullary carcinoma. With Romanowsky stains, the cytoplasmic granules of Hürthle cells are blue, whereas those of medullary carcinoma are usually red. Immunostains are helpful; Hürthle cells are thyroglobulin-positive and TTF-1-positive but calcitonin-negative.

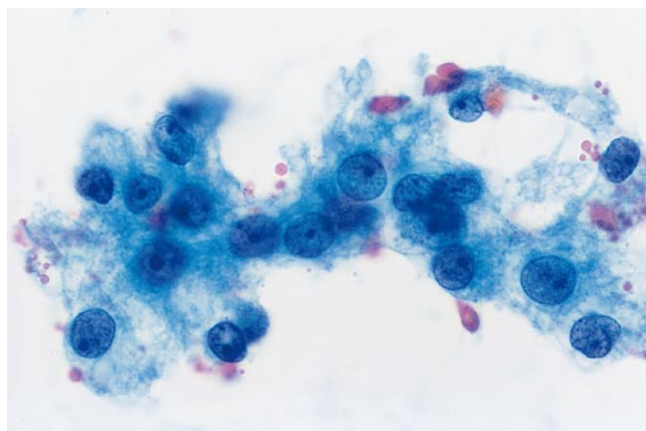


Figure 9.16 Metastatic renal cell carcinoma to the thyroid. The prominent nucleoli and abundant granular cytoplasm of renal cell carcinomas mimic the features of a Hürthle cell neoplasm (Papanicolaou stain).

MALIGNANT CONDITIONS

Papillary Carcinoma

Papillary carcinoma (PC) is the most common thyroid malignancy, accounting for 80% of all thyroid cancers in the United States.^{90,91} It can occur at any age, including in children, but most patients are between 20 and 50 years old. There is a female-to-male ratio of 4:1. PC presents as a solitary nodule or a distinct nodule within a nodular goiter. Some patients present with cervical lymphadenopathy resulting from a metastatic tumor.

PCs are defined histologically on the basis of their nuclear features. The nuclei are enlarged and can be oval, elongated, or irregular in contour. Profound changes in the nuclear skeleton make them less stiff and much more deformable than normal.⁹² The increased plasticity of the nucleus results in nuclear grooves (resulting from a nucleus folded on itself) and pseudoinclusions (from invagination of cytoplasm into the nucleus). The nuclei are paler than normal, but nuclear pallor may be patchy within the tumor. Nuclear crowding and overlapping is common. Papillary architecture (tumor cells arranged around a fibrovascular core) is seen in some but not all tumors. Focal squamous metaplasia is common. Some PCs are partly or predominantly cystic. Psammoma bodies are characteristic but not diagnostic of PC.

The classic PC has true papillary architecture, but a large number of “nonclassic” PC variants have been described. The most common is the *follicular variant*, which contains virtually no papillary structures; the *macrofollicular variant*, comprised predominantly of macrofollicles; the *oncocytic variant*, comprised of cells with abundant cytoplasm resembling Hürthle cells; the “*Warthin-like*” PC, with its abundant lymphoid infiltrate

and strong association with HT; the *clear cell variant* (self-explanatory); the *diffuse sclerosing variant*, which tends to occur in young adults and infiltrates in a diffuse rather than nodular pattern, with abundant squamous metaplasia and numerous psammoma bodies; the *tall cell variant*, whose cells are at least three times as tall as they are wide; the *columnar cell variant*, which has pseudostratified columnar cells; the *solid variant*, with solid growth but typical nuclear features of PC; the *cribriform carcinoma*, which occurs in patients with familial adenomatous polyposis and Gardner syndrome; and PC with *fasciitis-like stroma*. Additional variants that represent mixtures of PC and other thyroid cancers have been reported. Awareness of these variants is important for the cytopathologist who might otherwise confuse them with other neoplasms. Several of these, like the tall cell and columnar variants, have a tendency toward more aggressive clinical behavior than the classic PC.

PCs are immunoreactive for keratins, thyroglobulin, and TTF-1. Many PCs contain one of several chimeric oncogenes called *RET/PTC*, which result from gene rearrangements involving the *ret* proto-oncogene on chromosome 10.^{48,93} Other associated genetic changes include rearrangements of the *TRK* gene, point mutations of *RAS* proto-oncogenes, and point mutations of the *BRAF* gene.⁴⁸

Together with FC and HC, PC is considered one of the differentiated thyroid cancers. The prognosis for patients with PC is excellent because surgical resection (usually thyroidectomy) is often curative. The 10-year survival is over 90%. Many patients are treated with a so-called near-total thyroidectomy,⁹³ in which the lobe with the dominant tumor mass is entirely removed, the contralateral lobe is nearly completely removed, and a small amount of thyroid tissue is left to preserve the vascular supply to the parathyroid glands. More limited resections (e.g., unilateral lobectomy) result in a higher recurrence rate because many papillary carcinomas are multifocal.⁸²

CYTOMORPHOLOGY OF PAPILLARY CARCINOMA:

- sheets, papillae, microfollicles
- nuclear changes
 - “powdery” chromatin
 - grooves
 - pseudoinclusions
 - nucleolus (small or large)
 - membrane thickening and irregularity
- nuclear crowding or molding
- variable cytoplasm (scant, squamoid, Hürthle-like, or vacuolated)
- psammoma bodies
- histiocytes, including multinucleated giant cells

Typical architectural features of PC include papillae (Fig. 9.17) and sheets (Fig. 9.18). Microfollicles are seen in the follicular variant (Fig. 9.19). The diagnosis of PC does not rest on architectural but rather on nuclear changes. These include longitudinal grooves, intranuclear pseudoinclusions (“holes”), and, perhaps most significantly, a pale, “powdery” chromatin pattern (Fig. 9.20). In addition, nuclei are enlarged and crowded, and some may be molded to one another. A nucleolus is usually present and may be small or large. The cytoplasm can be inconspicuous or abundant and is not a helpful diagnostic feature. It can appear squamoid, oncocytic (Fig. 9.21), or vacuolated and thus is often misleading. In the diffuse sclerosing variant, for example, many of the tumor cells have a squamoid appearance.⁹⁴ *Multinucleated giant cells* are common in PC; they are CD68-positive histiocytes and not tumor giant cells.⁹⁵

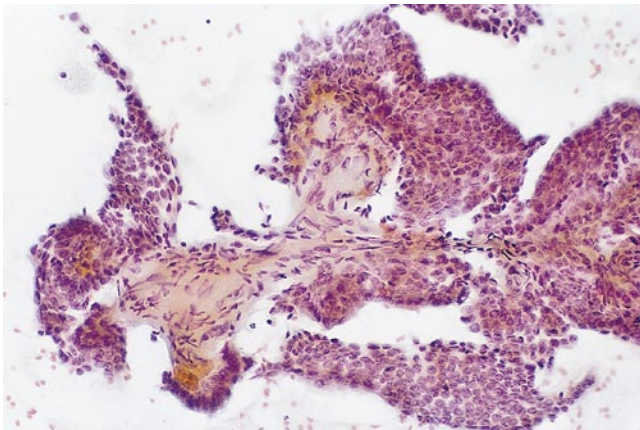


Figure 9.17 Papillary carcinoma (PC). In some cases, the tell-tale fibrovascular cores are seen (Papanicolaou stain).

Colloid is present and may be abundant. Sometimes it has an abnormal viscosity (“bubble gum colloid”) and may take the shape of long strands or dense blobs.

Psammoma bodies are a useful diagnostic feature (see Fig. 9.18). Although they are identified in about 50% of resected PCs,⁹¹ these concentric calcifications are less frequent on cytologic preparations and most numerous in the diffuse sclerosing variant.⁹⁴ They must be distinguished from non-specific, usually dystrophic calcifications, which are not laminated. Psammoma bodies should raise the suspicion of PC, especially in a cystic background, but because they are also seen in MNG⁹⁶ and Hürthle cell neoplasms,⁹¹ a conclusive diagnosis must be based on diagnostic cells. By themselves, the positive predictive value of psammoma bodies for PC is only 50%.⁹⁶

Cystic degeneration is especially common in PC and is a significant cause of false-negative diagnoses.⁹⁷ Macrophages, with or without hemosiderin, are the cytologic hallmark of cystic degeneration.

Some cases contain a population of enlarged “histiocytoid” tumor cells. They resemble histiocytes and have abundant vacuolated cytoplasm, but they are more atypical, with a larger and darker nucleus. They may or may not have nuclear grooves or inclusions. Rarely, such atypical (for PC) cells comprise the entire specimen. They are rarely seen in benign thyroid nodules and are thus relatively specific for PC.⁹⁸

In addition to the follicular variant of PC, two other variants deserve additional mention because of their distinctive cytologic features. The tall cell variant can be suspected when cells with PC nuclei have abundant, elongated, granular cytoplasm (Fig. 9.22). The columnar cell variant can be suspected when the cells are tall (columnar), with scant cytoplasm and crowded, cigar-shaped nuclei.

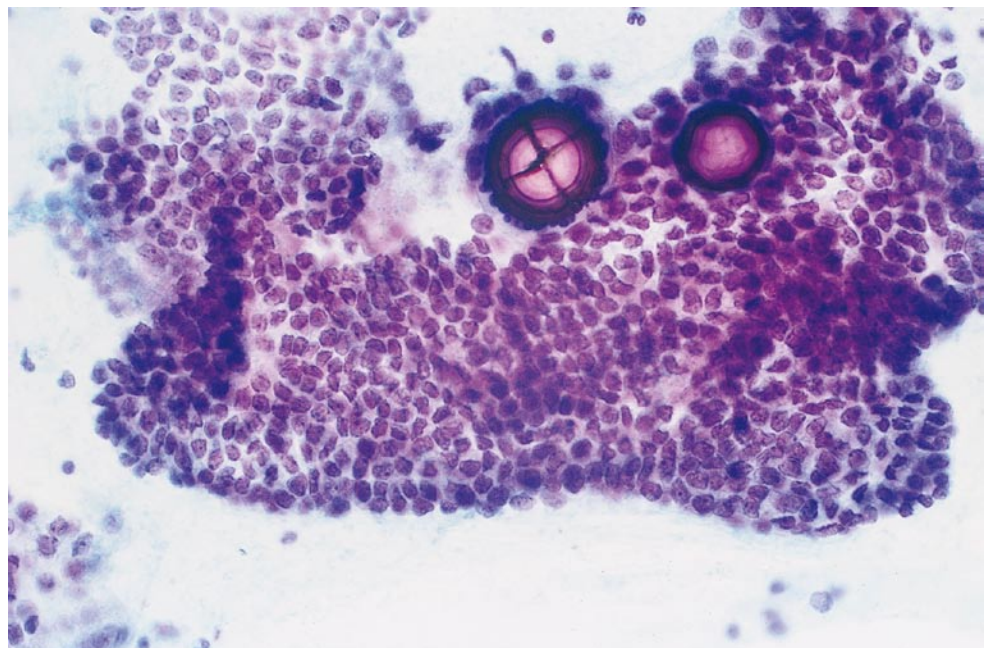


Figure 9.18 Papillary carcinoma (PC). In some cases, papillae are absent, and the neoplastic cells are arranged in crowded sheets. Psammoma bodies are present (Papanicolaou stain).

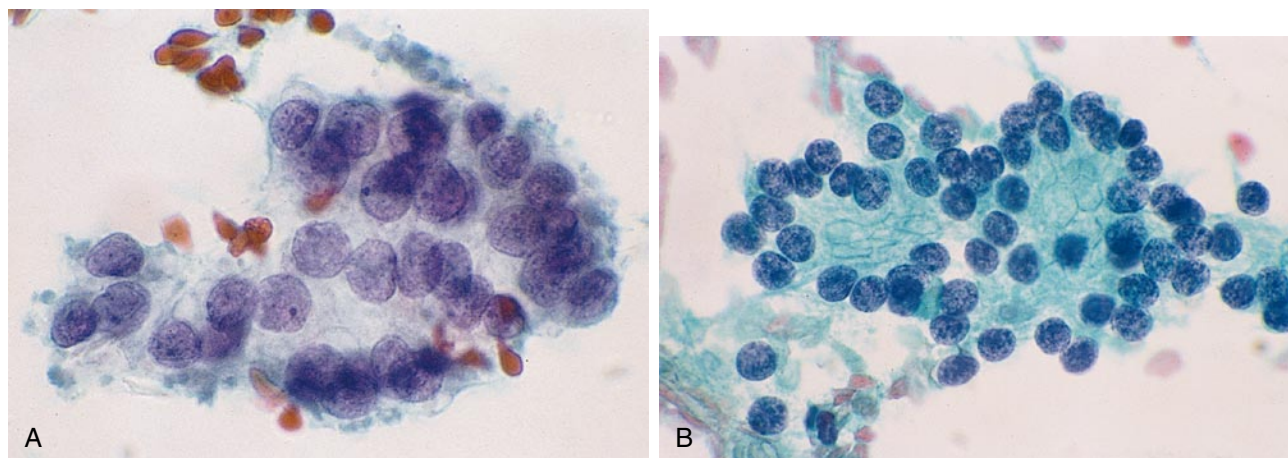


Figure 9.19 A, Papillary carcinoma (PC), follicular variant. B, Follicular neoplasm (follicular adenoma [FA]). Some PCs show microfollicular arrangements that mimic a follicular neoplasm, but the pale chromatin of a PC, A, is different from the coarse chromatin, granularity of a follicular neoplasm, B, (Papanicolaou stain).

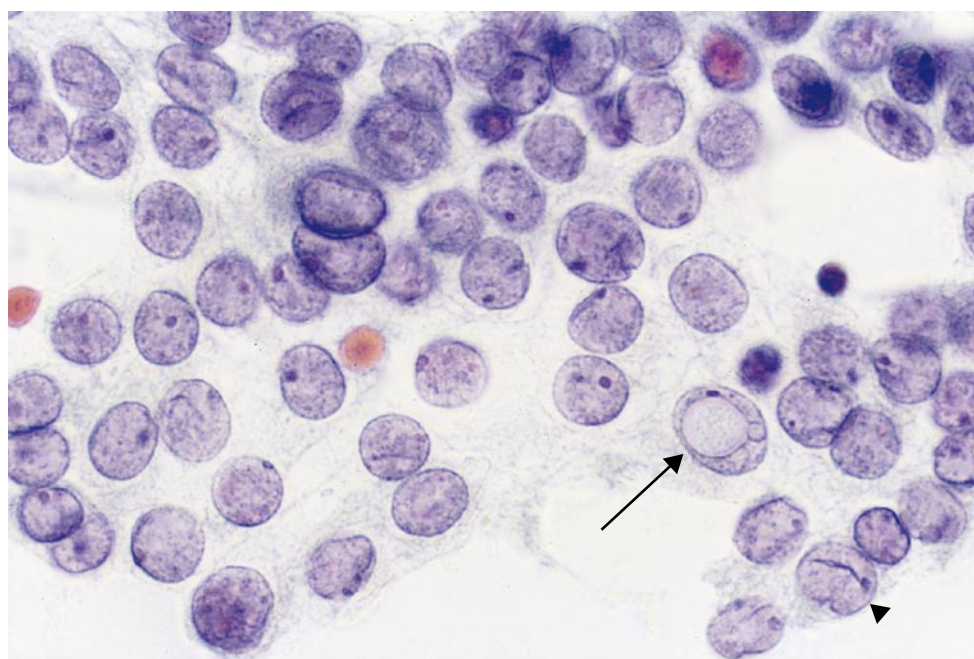


Figure 9.20 Papillary carcinoma (PC). The cells of PC have a characteristic pale, powdery chromatin. Other important nuclear changes are the circular, sharply defined intranuclear pseudoinclusion (arrow) and the nuclear groove (arrowhead). Note that the pseudoinclusion has the same color and consistency as the surrounding cytoplasm (Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF PAPILLARY CARCINOMA:

- benign follicular nodule
- follicular neoplasm
- HT
- ^{131}I treatment effect
- cyst lining cells
- hyalinizing trabecular tumor

The majority of PCs are straightforward to diagnose, because most or all of the nuclear and architectural changes previously described are clearly identifiable and widespread. Such cases are reliably interpreted as

malignant (see [Table 9.1](#)). In some PCs, however, the nuclear changes are subtle and focal. This is particularly true of the macrofollicular variant, which can be difficult to distinguish from a benign follicular nodule.⁶⁴ Other PCs may be incompletely sampled and yield only a small number of abnormal cells.⁹⁹ If only one or two characteristic features of PC are present, if they are only focal and not widespread throughout the follicular cell population, or if the sample is sparsely cellular, a malignant diagnosis cannot be made with certainty. Such cases occur with some regularity, and they are best classified as suspicious for malignancy, qualified as suspicious for papillary carcinoma (see [Table 9.1](#)).

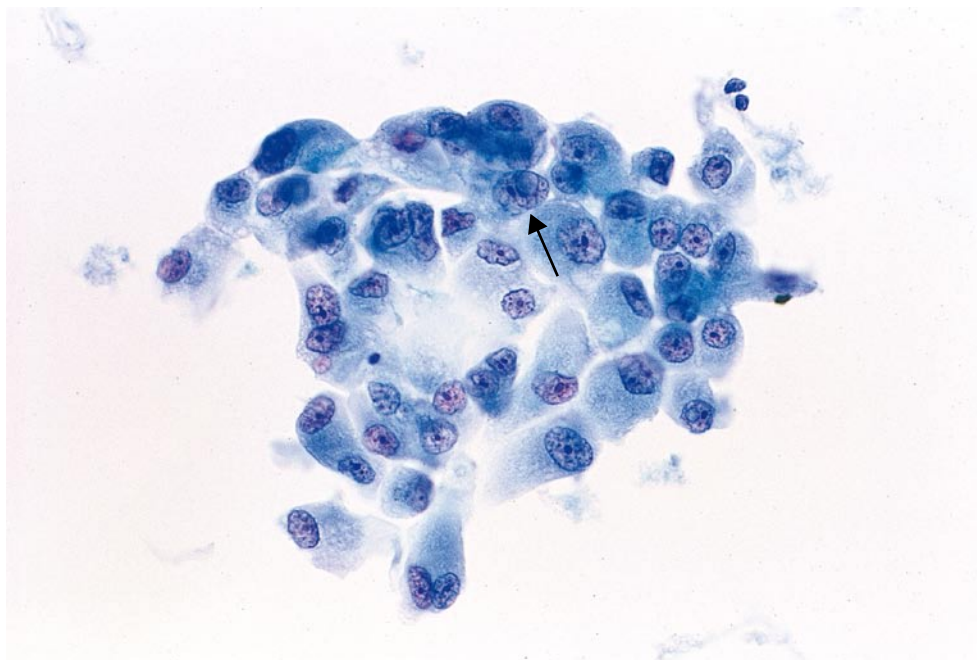


Figure 9.21 Papillary carcinoma (PC), oncocytic variant. Some PCs have abundant granular cytoplasm resembling Hürthle cells. The nuclear features in this case (arrow) are typical of PC (Papanicolaou stain).

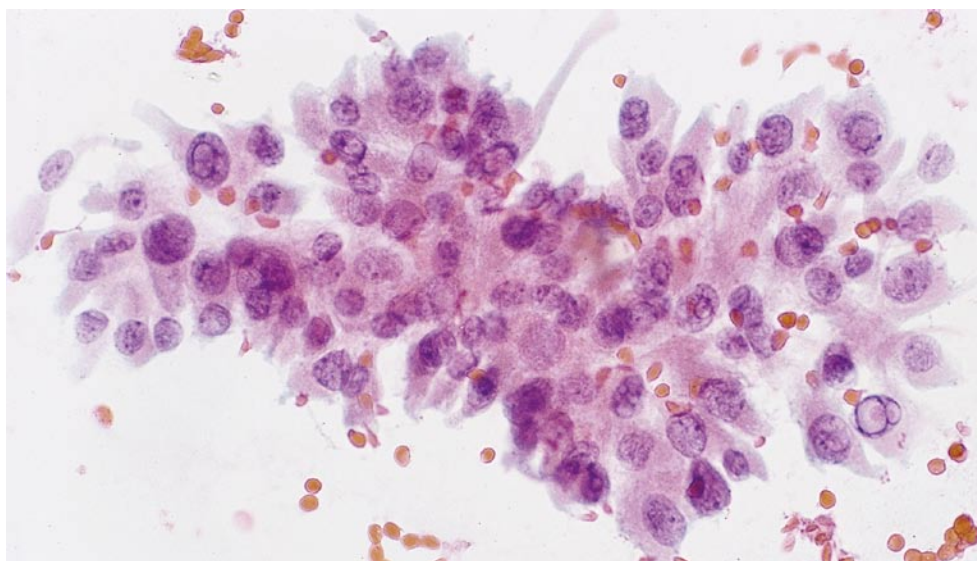


Figure 9.22 Papillary carcinoma (PC), tall cell variant. The cells are large and have abundant elongated ("tall") cytoplasm. Nuclear features typical of PC, including numerous intranuclear holes, are apparent (Papanicolaou stain).

Care must be taken not to overinterpret nonspecific findings as malignant or suspicious for PC. None of the features described above is diagnostic of PC by itself, and therefore a diagnosis (or even suspicion) of PC cannot be made on the basis of a single finding. If a well-sampled nodule has the characteristics of a benign follicular nodule, but a few grooves are present, they have little significance and can be ignored. The same applies to psammoma bodies, which occur not just in PC but also in MNG.¹⁰⁰ Beware of overinterpreting nuclear pallor that is not accompanied by other nuclear changes; it can result

from a technical staining problem. A variety of small nuclear holes, whether as a result of a fixation artifact or overlying red blood cells, mimic the pseudoinclusions of PC. Most nonspecific nuclear holes are easily recognized because they are small, not sharply etched, white (rather than the color of cytoplasm), and irregular in shape.

In patients with HT, some allowance needs to be made for mild nuclear atypia (e.g., focal pallor and occasional grooves). In small amounts, such changes are encountered with some regularity in thyroiditis, but they do not correlate with a histologic PC. In benign follicular nodules

affected by ^{131}I treatment there is usually hyperchromasia and marked nuclear size variation, which are not at all typical of PC. Cyst lining cells sometimes have large pale and grooved nuclei, and in cystic lesions they may be the predominant cell type. In such cases a suspicious for malignancy interpretation cannot be avoided.⁵⁰

The *hyalinizing trabecular tumor* (HTT) is a rare and controversial entity that may be a variant of PC. It has all the nuclear features of a PC, and most cases are interpreted as suspicious for PC or malignant by FNA.¹⁰¹ Cytologic clues to the diagnosis include a whorling, parallel array of elongated cells, amorphous hyaline material that is not amyloid, cytoplasmic “yellow bodies” (which stain light green with the Papanicolaou stain), and a perinucleolar clear zone, but most cases are identified by histologic, not cytologic, examination.¹⁰² Encapsulation, marked intratrabecular hyalinization, and trabecular architecture are important criteria. The discovery that some cases show rearrangement of *RET/PTC* has led some to hypothesize that HTTs are variants of PC.^{103,104} Others remain unconvinced and prefer to call them tumors or neoplasms rather than carcinomas.^{48,105}

Nodules called suspicious for papillary carcinoma are resected, either by lobectomy or thyroidectomy. Most (60% to 75%) prove to be PCs and the rest are usually FAs.^{11,12,38,106} Malignant nodules are usually removed by thyroidectomy. The positive predictive value of a malignant diagnosis is 97% to 99%.

Poorly Differentiated Carcinoma

Some carcinomas derived from follicular cells are not readily classified as one of the differentiated carcinomas (follicular, Hürthle cell, papillary) or as an undifferentiated (anaplastic) carcinoma. They fall somewhere in between, based on an intermediate degree of nuclear and architectural atypia. These tumors are called poorly differentiated carcinomas (PDCs) and account for 4% to 7% of thyroid carcinomas.^{48,107,108} Nodal, pulmonary, and bone metastases are common at the time of diagnosis. Patients with a PDC fare worse than those with one of the differentiated thyroid cancers, but better than those with an undifferentiated (anaplastic) carcinoma.⁹¹ The 5-year survival is about 50%.

The PDCs are a heterogeneous group, with three principal histologic patterns: insular, trabecular, and solid. In the insular pattern, the malignant cells are arranged in well-defined nests (“insulae”) surrounded by thin fibrovascular septae. Other PDCs have a predominantly trabecular (cords or ribbons) or solid growth pattern without nests. In all three patterns, some microfollicles (with or without colloid) can be seen. Tumor cells are generally small to intermediate in size and uniform, with some hyperchromasia, but pleomorphism is absent or only focal. Mitoses and necrosis are present. Nuclear features of PC are common, but some PDCs more closely resemble FC or HC.⁹¹ They are immunoreactive for thyroglobulin and TTF-1.

CYTOMORPHOLOGY OF POORLY DIFFERENTIATED CARCINOMA:

- highly cellular
- mostly single cells
- some microfollicles, trabeculae, spheres
- monomorphous round nuclei

The diagnosis of PDC rests on histologic evaluation and cannot be made with certainty by FNA. Nevertheless, some general comments can be made. Cytologic preparations are usually highly cellular (Fig. 9.23A), with numerous isolated tumor cells (Fig. 9.23B) and clusters of overlapping cells. Microfollicles are often present. In most cases the tumor cells are uniform and are the size of normal follicular cells,¹⁰⁷ but there may be a minor population of large pleomorphic cells as well.¹⁰⁸ Cytoplasm is poorly defined, and occasional cytoplasmic vacuoles are present. Intranuclear inclusions and nuclear grooves are present in some cases (Fig. 9.24).^{107,109} Colloid is scant. Although foci of necrosis are common in histologic sections, necrosis is generally not appreciated on cytologic preparations.

The differential diagnosis is broad. The presence of intranuclear inclusions and grooves in many of these tumors suggests a PC, but the prominence of isolated cells is unusual and might suggest PDC. Focal nuclear pleomorphism, if present, raises the possibility of an undifferentiated (anaplastic) carcinoma. Most notably, PDC has a striking resemblance to medullary carcinoma, but microfollicles with colloid support the diagnosis of PDC. Immunohistochemistry can be useful; PDCs are thyroglobulin-positive and calcitonin-negative.

A definite preoperative (i.e., FNA) diagnosis of PDC is not possible and can be made only with certainty after histologic examination.¹¹⁰ FNA specimens are usually diagnosed as follicular neoplasms^{109,110} or as metastatic carcinoma.¹¹¹

Undifferentiated (Anaplastic) Carcinoma

One of the most rapidly fatal of all cancers, undifferentiated (or anaplastic) carcinoma (UC) of the thyroid is uncommon and represents less than 5% of malignant thyroid tumors.⁴⁸ Despite its relatively low prevalence, it accounts for more than half of all deaths from thyroid cancer in the United States. Most patients are over the age of 60. Clinically, UC presents as a rapidly growing neck mass that has often spread to adjacent structures by the time it is diagnosed. Many patients present with hoarseness and dysphagia. Histologically, UCs are composed of spindle-shaped and epithelioid cells admixed with pleomorphic or osteoclast-type giant cells. Mitoses are numerous and extensive necrosis is common. Some cases have a prominent neutrophilic component. In about one third of cases, UC is associated with a

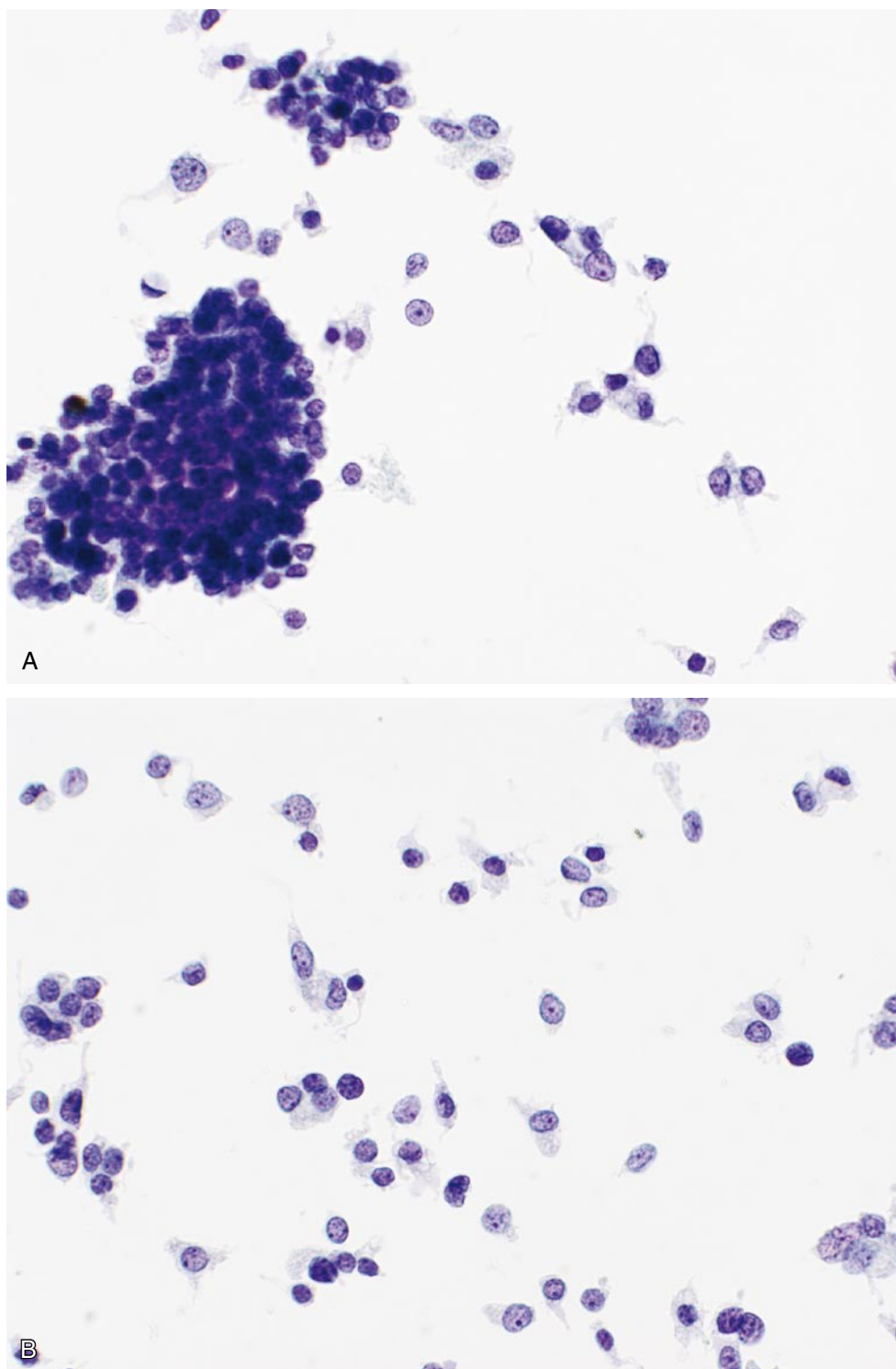


Figure 9.23 Poorly differentiated (insular) carcinoma (PDC). A, The architectural pattern is variable, and may include crowded groups, microfollicles, and numerous isolated cells (Papanicolaou stain). B, The isolated malignant cells resemble those of medullary carcinoma (Papanicolaou stain).

differentiated thyroid cancer like PC or FC, suggesting that many if not all UCs represent a dedifferentiation of a preexisting differentiated thyroid cancer.^{112, 113} UCs are usually reactive for keratins, but are negative for thyroglobulin and usually negative for TTF-1.¹¹⁴

By the time they are diagnosed, many UCs have extended beyond the thyroid into adjacent structures. Although surgery is often considered for palliation, complete excision is often impossible. Radiotherapy is of benefit in controlling local disease. The 5-year survival is 0% to 14%.

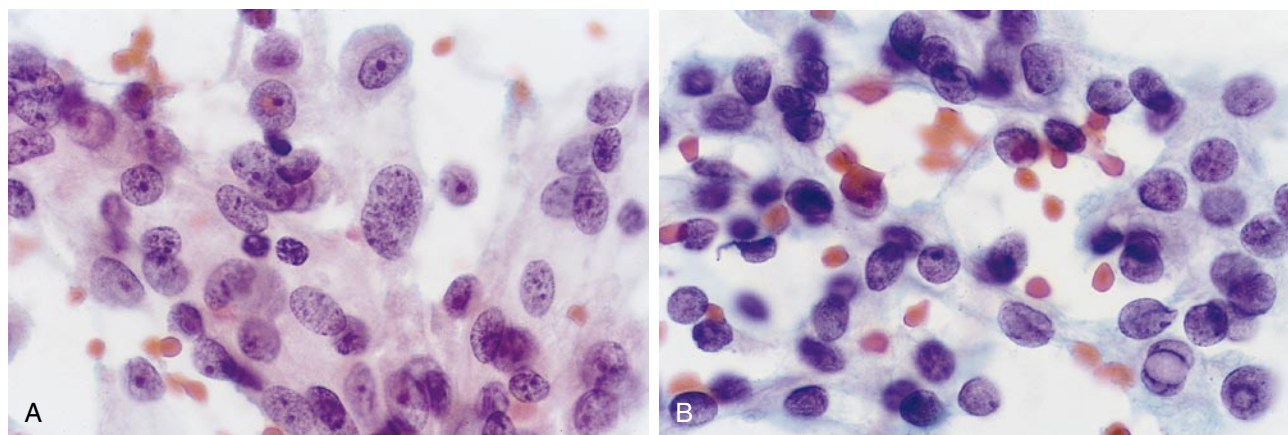


Figure 9.24 Poorly differentiated carcinoma (PDC). A, PDCs have a greater degree of nuclear atypia than the differentiated carcinomas (Papanicolaou stain). B, Nuclear changes of papillary carcinoma (e.g., pseudoinclusions) are often seen in PDCs (Papanicolaou stain).

CYTOMORPHOLOGY OF UNDIFFERENTIATED (ANAPLASTIC) CARCINOMA:

- mostly isolated cells
- marked nuclear pleomorphism
- large cells
- epithelioid or spindle shaped
- multinucleated giant cells
 - pleomorphic, tumor-type
 - osteoclast-type

The cytologic appearance is variable, reflecting the diversity of histologic patterns possible.¹¹⁵ The cells can be arranged as large fragments, small clusters, or isolated cells (Fig. 9.25). Often a tumor diathesis, including many necrotic cells, is noted. Tumor cells can be spindle-shaped or epithelioid, with squamoid features. Nuclei are hyperchromatic and vary widely in size and shape. Extremely large, pleomorphic, and bizarrely shaped cells are seen, along with multinucleated tumor giant cells. Some FNAs contain numerous osteoclast-type giant cells. The nuclear features are unmistakably malignant; the large, pleomorphic nuclei have irregular nuclear membranes, coarse and irregular chromatin clumping, and macronucleoli. Intranuclear inclusions are common, and mitotic figures may be numerous. In some cases, UC cells are accompanied by a distinct differentiated thyroid cancer component like PC.

DIFFERENTIAL DIAGNOSIS OF UNDIFFERENTIATED (ANAPLASTIC) CARCINOMA:

- atypia of cyst lining cells
- ¹³¹I treatment effect
- medullary carcinoma
- sarcoma
- metastatic carcinoma

MNGs and other benign follicular nodules with cystic degeneration contain a small number (usually) of atypical cells. Although the nuclei of these cyst lining cells are enlarged, they have smooth and regular nuclear membranes, finely granular chromatin, and small, regular nucleoli. Radioiodine effect can produce a wild variation in nuclear size of the affected follicular cells, but the pleomorphism of nuclear shape, mitotic activity, and necrosis of UC are not seen.

The differential diagnosis includes medullary carcinoma, sarcoma, and metastatic carcinoma to the thyroid. Sarcomas can be excluded with an immunohistochemical panel that includes cytokeratins, muscle markers (desmin, Myo-D1, myogenin), and vascular markers (CD31, CD34). Metastatic carcinoma is probably the most important consideration. Most UCs are immunoreactivity for keratins and negative for thyroglobulin and TTF-1.^{48,112,113} This immunoprofile is unhelpful in excluding metastatic carcinoma. The distinction rests heavily on clinical correlation and imaging studies; UC is ultimately a diagnosis of exclusion.

Squamous Cell Carcinoma

Squamous cell carcinoma (SQC) of the thyroid accounts for 1% or less of thyroid cancers. Like UC, it occurs in the elderly, and it has a similar dismal prognosis. Histologically, it is defined as a tumor with squamous differentiation throughout. Most are poorly differentiated. Cytologic preparations show pleomorphic keratinized cells (Fig. 9.26). The differential diagnosis includes undifferentiated carcinoma and metastatic SQC. Clinical correlation and imaging studies are critical for excluding a metastasis.

Medullary Thyroid Carcinoma

Medullary thyroid carcinoma (MTC) accounts for 5% to 10% of all thyroid carcinomas.⁴⁸ Unlike the previously discussed thyroid neoplasms, it arises not from the

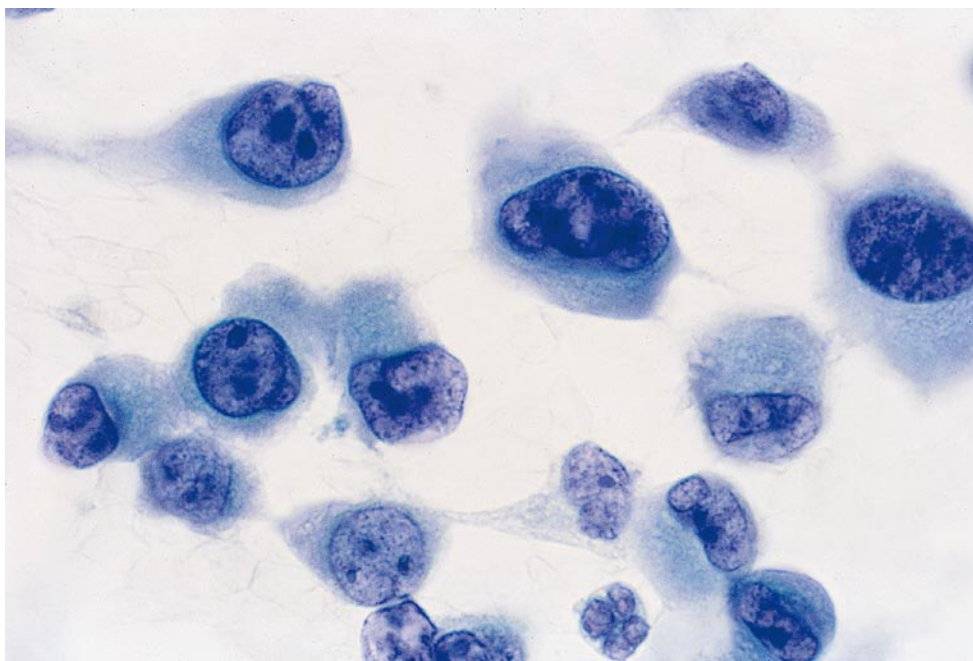


Figure 9.25 Undifferentiated (anaplastic) carcinoma (UC). Tumor cells are dispersed as isolated cells. Nuclei are large, hyperchromatic, and irregularly shaped (Papanicolaou stain).

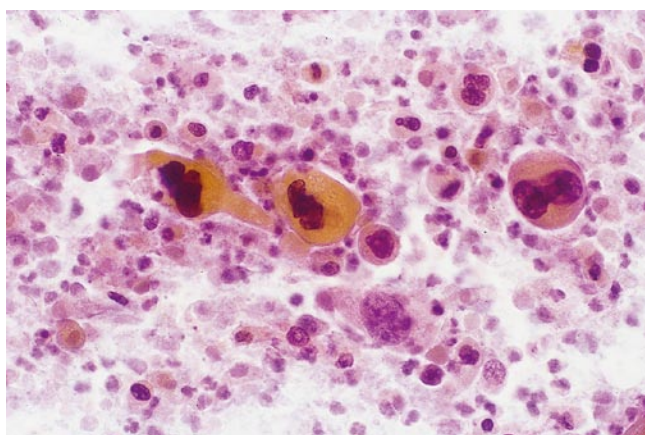


Figure 9.26 Squamous cell carcinoma (SQC). A pleomorphic malignancy in the thyroid that is comprised of keratinized cells is considered an SQC, but it has the same clinical behavior as an undifferentiated carcinoma (UC); Papanicolaou stain).

follicular cells but from the parafollicular cells, whose function is to synthesize and secrete calcitonin, the calcium-regulating hormone. About 80% to 90% are sporadic and occur in adults with a mean age of 50 years. The rest occur in children in association with genetic syndromes like the multiple endocrine neoplasia (MEN) syndromes. Patients with MEN 2a (Sipple syndrome) develop MTC and pheochromocytoma, sometimes with a hyperplasia or an adenoma of the parathyroid. Patients with MEN 2b (mucosal neuroma syndrome) have MTC, pheochromocytoma, multiple mucosal neuromas, and a marfanoid habitus. Because close to 90% of MTCs secrete calcitonin, the patient's serum can be used to screen for these tumors.

Up to 50% of patients with MTC present with regional node metastases. For this reason, MTC should be considered in the differential diagnosis of patients with a positive neck node and an unknown primary. Histologically, MTC is highly variable. Tumor cells are arranged in sheets, nests, or ribbons and can be polygonal, round, plasmacytoid, or spindle shaped. Nuclei are round or oval, and nucleoli are usually not prominent. Intranuclear pseudoinclusions, indistinguishable from those in PC, are seen. Occasional cells can be markedly enlarged and pleomorphic. Amyloid deposits are seen in 80% of cases and can be confirmed with the Congo red stain. MTCs are immunoreactive for calcitonin, TTF-1, carcinoembryonic antigen (CEA), and chromogranin; they are negative for thyroglobulin.

MTCs are usually treated with total thyroidectomy with excision of regional lymph nodes.⁹³

CYTOMORPHOLOGY OF MEDULLARY THYROID CARCINOMA:

- numerous isolated cells
- loose clusters
- epithelioid, plasmacytoid, or spindle-shaped cells
- nuclei
 - round or elongated
 - granular chromatin
 - inconspicuous nucleolus
 - pseudoinclusions (50% of cases)
 - multiple nuclei
- red cytoplasmic granules (70% of cases)
- amyloid

The cytologic picture is variable.¹¹⁶ Tumor cells are predominantly isolated (Fig. 9.27A), but some loose clusters and rosettes are seen (Fig. 9.27B). They are usually uniform in size and shape, but many cases contain at least a few alarmingly large cells. Cytoplasm is moderate or abundant and finely granular. Red cytoplasmic granules are observed with Romanowsky-type stains in some, but not all cases¹¹⁶ (see Fig. 9.27B). Nuclei are eccentrically placed, which gives these cells a plasmacytoid appearance, and binucleation and multinucleation are common. In some cases the cells are decidedly spindle-shaped, and the eccentrically placed nucleus makes the cells look like a comet with a long cytoplasmic tail. Nuclei have a coarsely granular, “salt-and-pepper” chromatin texture and inconspicuous nucleoli. Intranuclear inclusions are found in about one half of cases.¹¹⁶ Amyloid is present in most but not all cases (Fig. 9.27A). It is similar to colloid but can be distinguished with the Congo red stain, which shows apple-green dichroism with polarized light.

DIFFERENTIAL DIAGNOSIS OF MEDULLARY THYROID CARCINOMA:

- UC
- Hürthle cell neoplasm
- PC
- PDC
- metastatic tumor

The large, pleomorphic cell component of some MTCs raises the possibility of a UC, but the more prominent well-differentiated component of MTCs is not typical of UC. A Hürthle cell neoplasm is a frequent consideration, because the cells of both neoplasms are often dispersed as isolated cells with moderate to abundant cytoplasm. But macronucleoli are rarely seen in MTC, and Hürthle cell neoplasms do not have salt-and-pepper chromatin. The color of the cytoplasmic granules with the Romanowsky-type stains can provide an additional clue: if red, the tumor is more likely to be an MTC. PC and MTC have intranuclear inclusions, but other nuclear and architectural features help distinguish the two. MTC and PDC can be difficult to distinguish by cytomorphology alone. The same applies for certain metastatic tumors, particularly melanoma. Immunohistochemistry is helpful to confirm the diagnosis of MTC (see Fig. 9.27C). Virtually all cases of MTC are positive for calcitonin, TTF-1, chromogranin, and CEA. If immunohistochemistry cannot be performed, a serum calcitonin level can be obtained. Calcitonin is elevated in virtually all patients with MTC.⁴⁸

Lymphoma

Primary thyroid non-Hodgkin lymphoma (PTNHL) represents 5% of thyroid cancers.⁴⁸ PTNHLs typically occur in older-aged women, and virtually all arise in the setting of HT. The relative risk for a patient with Hashimoto's thyroiditis is about 40 to 80:1 compared with the general population, but PTNHL is still a rare complication. When it does occur, it is usually 20 to 30 years after the onset of HT, and the mean age of patients is 65 years. Most patients present with a noticeably enlarging mass, and compressive symptoms (dyspnea, dysphagia, hoarseness) occur in about one third of patients.¹¹⁷ In some patients, cervical lymph nodes are involved. The average size of a PTNHL is 7 cm, but tumors as big as 20 cm have been reported.¹¹⁷

PTNHLs fall into three general categories: extra-nodal marginal zone B-cell lymphoma (MZL); diffuse large B-cell lymphoma (DLBL); and mixed MZL/DLBL type. Other types occur but are distinctly less common.^{48,117}

CYTOMORPHOLOGY OF THYROID LYMPHOMAS:

Extra-nodal marginal zone B-cell lymphoma

- small lymphoid cells
 - centrocytes
 - plasma cells
 - monocytoid B cells
- interspersed large lymphoid cells

Diffuse large B-cell lymphoma

- large lymphoid cells
- centroblasts
- immunoblasts
- Burkitt-like cells

Cytologic preparations are highly cellular and composed of isolated lymphoid cells (Fig. 9.28). Lymphoepithelial lesions, characteristic of MZLs, are difficult to identify in smears.

A lymphoma should be suspected in any patient with longstanding thyroiditis and a large or enlarging thyroid mass. The main differential diagnosis is HT. PTNHLs that have a significant component of DLBL are the easiest to distinguish from HT because of the large, highly atypical lymphocytes. MZL is more difficult to distinguish cytomorphologically from HT. For this reason, demonstration of clonal restriction (by flow cytometry, immunocytochemistry, or molecular diagnostic methods) can be useful when lymphoma is suspected on clinical or cytomorphologic grounds. Caution is advised, because some HT specimens contain clonal B-cell populations that do not equate to

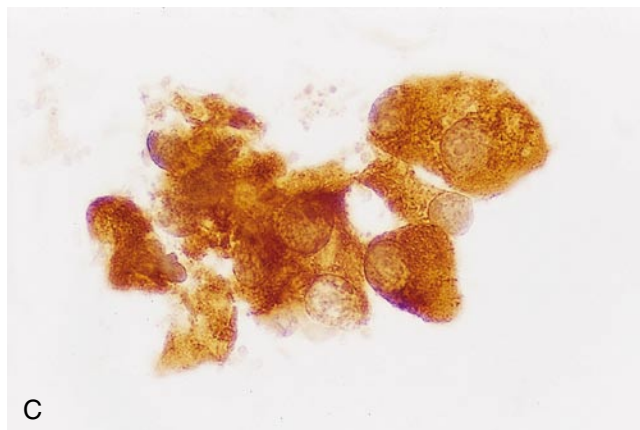
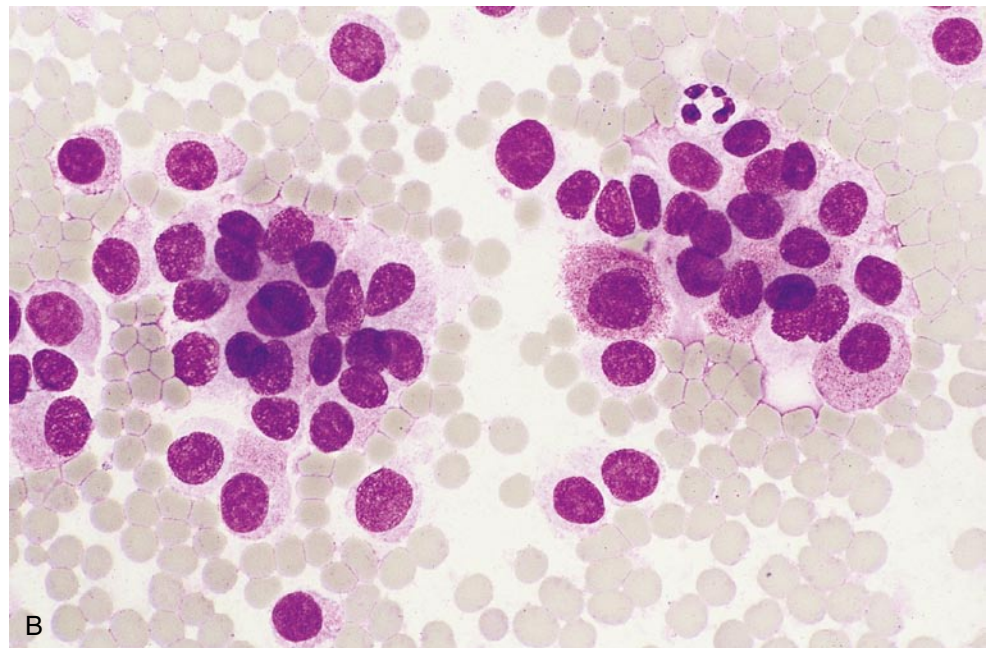
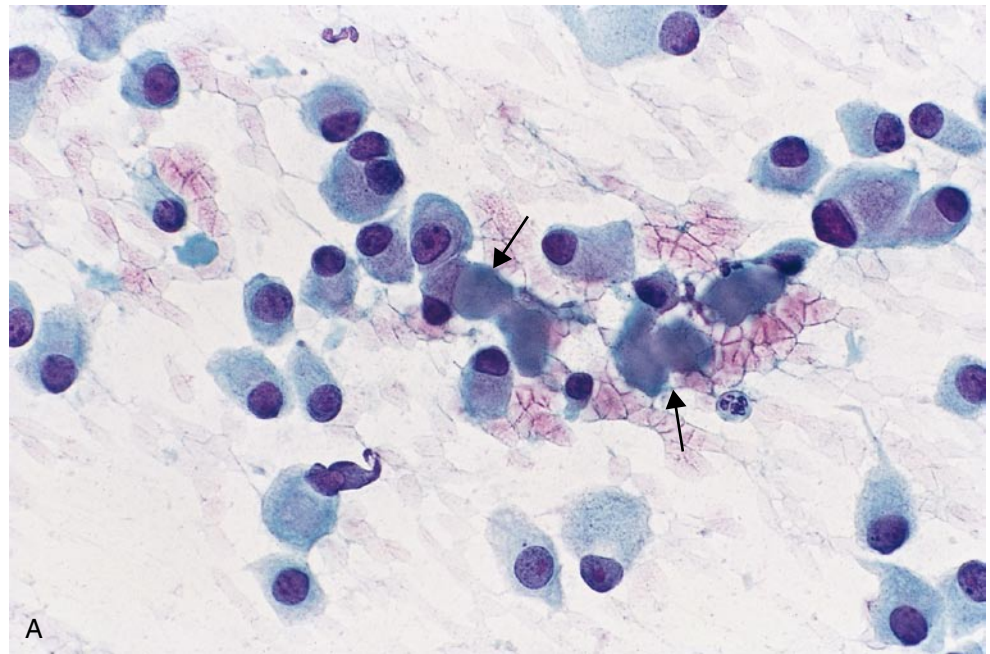


Figure 9.27 Medullary carcinoma. A, Smears show numerous isolated cells and small blobs of amyloid (arrows; Papanicolaou stain). B, Air-dried Romanowsky-stained preparations show fine red cytoplasmic granules, a helpful diagnostic feature. (Courtesy of Dr. James Cappellari IV, Wake Forest University, Winston-Salem, NC, USA.) C, The malignant cells are strongly immunoreactive for calcitonin.

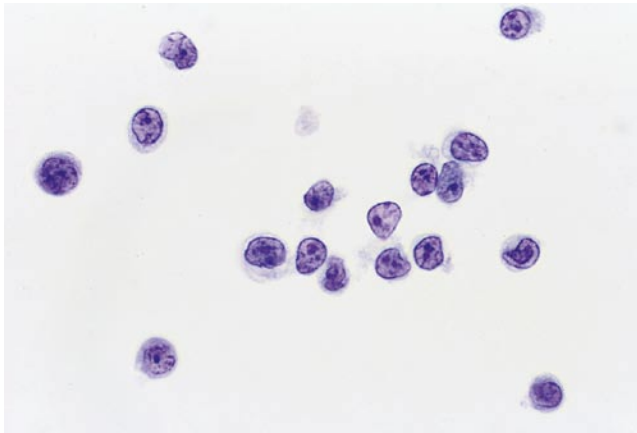


Figure 9.28 Extranodal marginal zone B-cell lymphoma of the thyroid. The neoplastic lymphoid cells are uniformly small, with irregularly shaped nuclei and a moderate amount of cytoplasm (Papanicolaou stain).

malignancy and are thus misleading.^{67,68} For this reason, immunophenotyping or molecular genetics are recommended only if the clinical presentation (rapid growth, large nodule) or cytomorphology (monomorphous or atypical lymphoid population) suggest lymphoma. If immunophenotyping or molecular diagnostics are not available and the cytomorphology is equivocal, the interpretation suspicious for malignancy, qualified as suspicious for non-Hodgkin lymphoma might be most appropriate (see Table 9.1). In such cases, a repeat FNA with allocation of cells for flow cytometry or molecular studies can be considered.

Metastatic Carcinoma

Metastatic tumors to the thyroid are encountered in 0.1% to 0.3% of thyroid aspirates,^{118,119} the lungs, esophagus, breast, and kidney are the most common primary sites.¹²⁰ The possibility of a metastasis should be considered whenever the cytologic picture does not conform with common thyroid neoplasms or when the patient has a history of cancer elsewhere in the body. A clinical history of a known malignancy should be provided on the requisition.¹⁴ In 25% to 50% of cases, however, there is no previous history of malignancy, and the thyroid metastasis is the first manifestation of an occult malignancy.^{118,119} The distinction between a primary thyroid cancer and a metastasis can be difficult. Some lung cancers can mimic undifferentiated and SQCs of the thyroid; melanoma can mimic medullary thyroid carcinoma; and metastatic clear cell carcinoma of the kidney is a particularly good mimic of a follicular or Hürthle cell neoplasm (see Fig. 9.16).⁸⁴ Comparing the aspirated specimen to any previous histologic or cytologic material is helpful, as is immunohistochemistry for thyroglobulin, calcitonin, and TTF-1.

PARATHYROID TUMORS

Parathyroid adenomas and the rare parathyroid carcinoma can be mistaken clinically for thyroid nodules. Smears are cellular, composed of cohesive sheets, ribbon-like cords, and occasional microacini (Fig. 9.29).

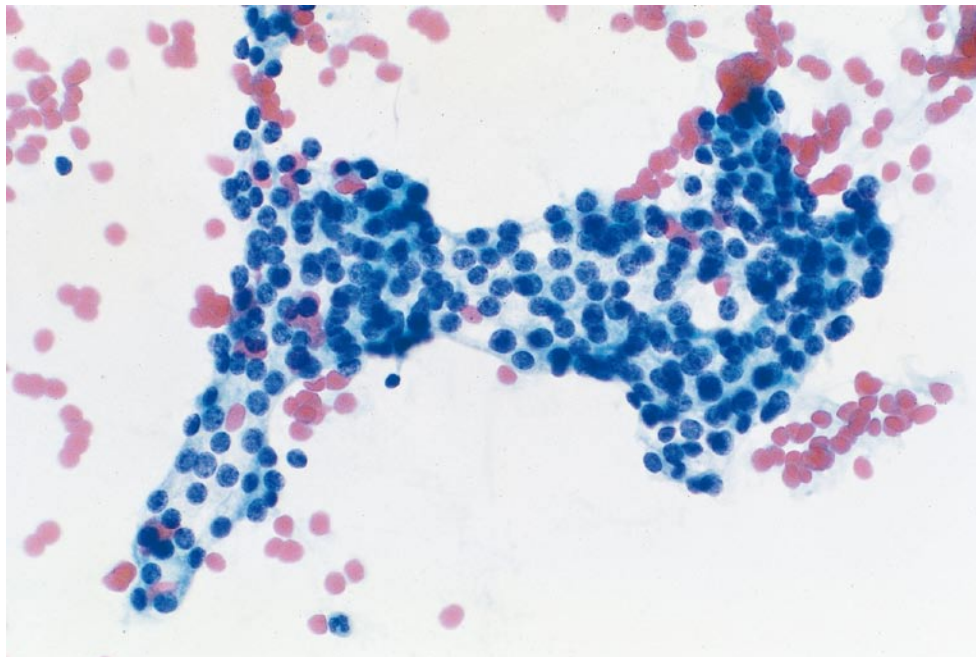


Figure 9.29 Parathyroid adenoma. Smears are often highly cellular, and the cells are arranged in groups that resemble macrofollicle fragments or as seen here, crowded trabeculae.

Nuclei are round and have a coarsely granular chromatin pattern; nucleoli are small or prominent. Cytoplasm is moderately abundant and granular. Isolated cells and naked nuclei can be present. Focal nuclear pleomorphism is seen, but it is not prominent. Colloid is absent.

Parathyroid adenomas are frequently mistaken cytologically for a follicular lesion of the thyroid.^{121,122} Most patients with a parathyroid adenoma have hypercalcemia, which is a clue to the correct diagnosis. Immunohistochemistry for thyroglobulin and parathyroid hormone can be helpful if a nonthyroid origin is suspected.

REFERENCES

1. Stoffer RP, Welch JW, Hellwig CA, et al.: Nodular goiter: Incidence, morphology before and after iodine prophylaxis, and clinical diagnosis. *Arch Intern Med* 1960;106:10-14.
2. Vander JB, Gaston EA, Dawber TR: The significance of nontoxic thyroid nodules: Final report of a 15-year study of the incidence of thyroid malignancy. *Arch Intern Med* 1968;69:537-540.
3. Ezzat S, Sarti DA, Cain DR, Braunstein GD: Thyroid incidentalomas: prevalence by palpation and ultrasonography. *Arch Intern Med* 1994;154:1838-1840.
4. Mortensen JD, Woolner LB, Bennett WA: Gross and microscopic findings in clinically normal thyroid glands. *J Clin Endocrinol Metab* 1955;15:1270-1280.
5. Brander A, Viikinkoski P, Nickels J, Kivisaari L: Thyroid gland: US screening in a random adult population. *Radiology* 1991;181(3):683-687.
6. Bruneton JN, Balu-Maestro C, Marcy PY, et al.: Very high frequency (13 MHz) ultrasonographic examination of the normal neck: Detection of normal lymph nodes and thyroid nodules. *J Ultrasound Med* 1994;13(2):87-90.
7. Horlocker TT, Hay JE, James EM, et al.: Prevalence of incidental nodular thyroid disease detected during high-resolution parathyroid ultrasonography. In Medeiros-Neto G, Gaitan E (eds): *Frontiers in Thyroidology*. New York, Plenum Medical Book Co, 1986, pp. 1309-1312.
8. Galloway JW, Sardi A, DeConti RW, et al.: Changing trends in thyroid surgery: 38 years' experience. *Am Surg* 1991;57:18-20.
9. Hamberger B, Gharib H, Melton LJ 3rd, et al.: Fine-needle aspiration biopsy of thyroid nodules: Impact on thyroid practice and cost of care. *Am J Med* 1982;73:381-384.
10. Suen KC: How does one separate cellular follicular lesions of the thyroid by fine-needle aspiration biopsy? *Diagn Cytopathol* 1988;4:78-81.
11. Yang J, Schnadig V, Logrono R, Wasserman PG: Fine-needle aspiration of thyroid nodules: A study of 4703 patients with histologic and clinical correlations. *Cancer* 2007;111(5):306-315.
12. Yassa L, Cibas ES, Benson CB, et al.: Long-term assessment of a multidisciplinary approach to thyroid nodule diagnostic evaluation. *Cancer* 2007;111(6):508-516.
13. Marqusee E, Benson CB, Frates MC, et al.: Usefulness of ultrasonography in the management of nodular thyroid disease. *Ann Intern Med* 2000;133:696-700.
14. Cibas ES, Alexander EK, Benson CB, et al.: Indications for Thyroid FNA and Pre-FNA Requirements: A Synopsis of the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference Diagnostic Cytopathology 2008; 36:390-399.
15. Ashcraft MW, Van Herle AJ: Management of thyroid nodules. II: Scanning techniques, thyroid suppressive therapy, and fine needle aspiration. *Head Neck Surg* 1981;3(4):297-322.
16. Hegedus L, Bonnema SJ, Bennedbaek FN: Management of simple nodular goiter: Current status and future perspectives. *Endocr Rev* 2003;24(1):102-132.
17. Frates MC, Benson CB, Charboneau JW, et al.: Management of thyroid nodules detected at US: Society of Radiologists in Ultrasound consensus conference statement. *Radiology* 2005;237(3):794-800.
18. Danese D, Sciacchitano S, Farsetti A, et al.: Diagnostic accuracy of conventional versus sonography-guided fine-needle aspiration biopsy of thyroid nodules. *Thyroid* 1998;8:15-21.
19. Hatada T, Okada K, Ishii H, et al.: Evaluation of ultrasound-guided fine-needle aspiration biopsy for thyroid nodules. *Am J Surg* 1998;133-136.
20. Takashima S, Fukuda H, Kabayashi T: Thyroid nodule: Clinical effect of ultrasound-guided fine-needle aspiration biopsy. *J Clin Ultrasound* 1994;22:535-542.
21. Pitman MB, Abele J, Ali S, et al.: Techniques for thyroid FNA: A synopsis of the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. *Diagnostic Cytopathology* 2008; 36:407-424.
22. Eedes CR, Wang HH: Cost-effectiveness of immediate specimen adequacy assessment of thyroid fine-needle aspirations. *Am J Clin Pathol* 2004;121(1):64-69.
23. Gordon DL, Gattuso P, Castelli M, et al.: Effect of fine needle aspiration biopsy on the histology of thyroid neoplasms. *Acta Cytol* 1993;37:651-654.
24. Baloch ZW, LiVolsi VA: Post fine-needle aspiration histologic alterations of thyroid revisited. *Am J Clin Pathol* 1999; 112(3):311-316.
25. Hastei F, Pang Y, Pu R, Michael CW: Do we need more than one ThinPrep to obtain adequate cellularity in fine needle aspiration? *Diagn Cytopathol* 2007;35(11):740-743.
26. Tulecke MA, Wang HH: ThinPrep for cytologic evaluation of follicular thyroid lesions: Correlation with histologic findings. *Diagn Cytopathol* 2004;30(1):7-13.
27. Biscotti CV, Hollow JA, Toddy SM, Easley KA: ThinPrep versus conventional smear cytologic preparations in the analysis of thyroid fine-needle aspiration specimens. *Am J Clin Pathol* 1995;104:150-153.
28. Frost AR, Sidawy MK, Ferfelli M, et al.: Utility of thin-layer preparations in thyroid fine-needle aspiration. Diagnostic accuracy, cytomorphology, and optimal sample preparation. *Cancer (Cancer Cytopathol)* 1998;84:17-25.
29. Scurry JP, Duggan MA: Thin layer compared to direct smear in thyroid fine needle aspiration. *Cytopathology* 2000;11(2): 104-115.
30. Malle D, Valeri RM, Pazaitou-Panajiotou K, et al.: Use of a thin-layer technique in thyroid fine needle aspiration. *Acta Cytol* 2006;50(1):23-27.
31. Hoda RS: Non-gynecologic cytology on liquid-based preparations: A morphologic review of facts and artifacts. *Diagn Cytopathol* 2007;35(10):621-634.
32. Goellner JR, Gharib H, Grant CS, Johnson DA: Fine-needle aspiration cytology of the thyroid, 1980 to 1986. *Acta Cytol* 1987;31:587-590.
33. Grant CS, Hay ID, Gough IR, et al.: Long-term follow-up of patients with benign thyroid fine-needle aspiration cytologic diagnoses. *Surgery* 1989;106:980-985.
34. Hamburger JI, Husain M: Semiquantitative criteria for fine-needle biopsy diagnosis: Reduced false-negative diagnoses. *Diagn Cytopathol* 1988;4:14-17.
35. Ravetto C, Colombo L, Dottorini ME: Usefulness of fine-needle aspiration in the diagnosis of thyroid carcinoma: A retrospective study in 37,895 patients. *Cancer* 2000;90:357-363.
36. Renshaw AA: Accuracy of thyroid fine-needle aspiration using receiver operator characteristic curves. *Am J Clin Pathol* 2001;116:477-482.

37. Amrikachi M, Ramzy I, Rubinfeld S, Wheeler TM: Accuracy of fine-needle aspiration of thyroid: A review of 6226 cases and correlation with surgical or clinical outcome. *Arch Pathol Lab Med* 2001;125:484-488.
38. Gharib H, Goellner JR, Johnson DA: Fine-needle aspiration cytology of the thyroid: A 12-year experience with 11,000 biopsies. *Clin Lab Med* 1993;13:699-709.
39. McHenry CR, Walfish PG, Rosen IB: Non-diagnostic fine-needle aspiration biopsy: A dilemma in management of nodular thyroid disease. *Am Surg* 1993;59:415-419.
40. van Hoesen KH, Gupta PK, LiVolsi VA: Value of repeat fine needle aspiration (FNA) of the thyroid [Abstract]. *Mod Pathol* 1994;7:43A.
41. Layfield L, Cochand-Priollet B, LiVolsi V, et al.: Post thyroid FNA testing and treatment options: A synopsis of the National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. *Diagnostic Cytopathology* 2008.
42. Gharib H, Goellner JR: Fine-needle aspiration biopsy of the thyroid: an appraisal. *Ann Int Med* 1993;118:282-289.
43. Kini SR: Thyroid. In Kline TS (ed): *Guides to Clinical Aspiration Biopsy Series*, 2nd ed. New York, Igaku-Shoin, 1996.
44. Khurana KK, Baloch ZW, LiVolsi VA: Aspiration cytology of pediatric solitary papillary hyperplastic thyroid nodule. *Arch Pathol Lab Med* 2001;125(12):1575-1578.
45. Jayaram G: Respiratory epithelial cells in fine-needle aspirates of thyroid [Letter]. *Acta Cytol* 1995;39:834-835.
46. Day TA, Chu A, Hoang KG: Multinodular goiter. *Otolaryngol Clin North Am* 2003;36(1):35-54.
47. Krohn K, Fuhrer D, Bayer Y, et al.: Molecular pathogenesis of euthyroid and toxic multinodular goiter. *Endocr Rev* 2005;26(4):504-524.
48. DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds): *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Endocrine Organs*. Lyon, France, IARC Press, 2004.
49. Harach HR, Zusman SB, Saravia Day E: Nodular goiter: a histocytological study with some emphasis on pitfalls of fine-needle aspiration cytology. *Diagn Cytopathol* 1992;8(4):409-419.
50. Faquin WC, Cibas ES, Renshaw AA: "Atypical" cells in fine-needle aspiration biopsy specimens of benign thyroid cysts. *Cancer* 2005;105(2):71-79.
51. Luck JB, Mumaw VR, Frable WJ: Fine needle aspiration biopsy of the thyroid: Differential diagnosis by Videoplan image analysis. *Acta Cytol* 1982;26:793-796.
52. Wright RG, Castles H, Mortimer RH: Morphometric analysis of thyroid cell aspirates. *J Clin Pathol* 1987;40:443-445.
53. Boon ME, Lowhagen T, Willems JS: Planimetric studies on fine needle aspirates from follicular adenoma and follicular carcinoma of the thyroid. *Acta Cytol* 1980;24:145-148.
54. Cochand-Priollet B, Koutroumbas K, Megalopoulou TM, et al.: Discriminating benign from malignant thyroid lesions using artificial intelligence and statistical selection of morphometric features. *Oncol Rep* 2006;15 Spec no.:1023-1026.
55. Crissman JD, Drozdowicz S, Johnson C, Kini SR: Fine needle aspiration diagnosis of hyperplastic and neoplastic follicular nodules of the thyroid: A morphometric study. *Anal Quant Cytol Histol* 1991;13:321-328.
56. Salmon I, Gasperin P, Pasteels JL, et al.: Relationship between histopathologic typing and morphonuclear assessments of 238 thyroid lesions: digital cell image analysis performed on Feulgen-stained nuclei from formalin-fixed, paraffin-embedded materials. *Am J Clin Pathol* 1992;97:776-786.
57. Grant CS, Hay ID, Ryan JJ, et al.: Diagnostic and prognostic utility of flow cytometric DNA measurements in follicular thyroid tumors. *World J Surg* 1990;14:283-290.
58. Joensuu H, Klemi PJ: DNA aneuploidy in adenomas of endocrine organs. *Am J Pathol* 1988;132:145-151.
59. Klemi PJ, Joensuu H, Eerola E: DNA aneuploidy in anaplastic carcinoma of the thyroid gland. *Am J Clin Pathol* 1988;89:154-159.
60. Schelfhout LJ, Cornelisse CJ, Gaslings BM, et al.: Frequency and degree of aneuploidy in benign and malignant thyroid neoplasms. *Int J Cancer* 1990;45:16-20.
61. Rickert D, Mittermayer C, Lindenfelser R, Biesterfeld S: MIB-1 immunohistochemistry of follicular adenoma and follicular carcinoma of the thyroid gland. *Anal Quant Cytol Histol* 2000;22:229-234.
62. Bartolazzi A, Gasbarri A, Papotti M, et al.: Application of an immunodiagnostic method for improving preoperative diagnosis of nodular thyroid lesions. *Lancet* 2001;357:1644-1650.
63. French CA, Alexander EK, Cibas ES, et al.: Genetic and biological subgroups of low-stage follicular thyroid cancer. *Am J Pathol* 2003;162(4):1053-1060.
64. Chung D, Ghossein RA, Lin O: Macrofollicular variant of papillary carcinoma: A potential thyroid FNA pitfall. *Diagn Cytopathol* 2007;35(9):560-564.
65. Nassar A, Gupta P, LiVolsi VA, Baloch Z: Histiocytic aggregates in benign nodular goiters mimicking cytologic features of papillary thyroid carcinoma (PTC). *Diagn Cytopathol* 2003;29(5):243-245.
66. Holm LE, Blomgren H, Lowhagen T: Cancer risks in patients with chronic lymphocytic thyroiditis. *N Engl J Med* 1985;312(10):601-604.
67. Saxena A, Alport EC, Moshynska O, et al.: Clonal B cell populations in a minority of patients with Hashimoto's thyroiditis. *J Clin Pathol* 2004;57(12):1258-1263.
68. Chen HI, Akpolat I, Mody DR, et al.: Restricted kappa/lambda light chain ratio by flow cytometry in germinal center B cells in Hashimoto thyroiditis. *Am J Clin Pathol* 2006;125(1):42-48.
69. Fatourechi V, Aniszewski JP, Fatourechi GZ, et al.: Clinical features and outcome of subacute thyroiditis in an incidence cohort: Olmsted County, Minnesota, study. *J Clin Endocrinol Metab* 2003;88(5):2100-2105.
70. Benbassat CA, Olchovsky D, Tsvetov G, Shimon I: Subacute thyroiditis: Clinical characteristics and treatment outcome in fifty-six consecutive patients diagnosed between 1999 and 2005. *J Endocrinol Invest* 2007;30(8):631-635.
71. Shabb NS, Salti I: Subacute thyroiditis: Fine-needle aspiration cytology of 14 cases presenting with thyroid nodules. *Diagn Cytopathol* 2006;34(1):18-23.
72. Shabb NS, Tawil A, Gergeos F, et al.: Multinucleated giant cells in fine-needle aspiration of thyroid nodules: Their diagnostic significance. *Diagn Cytopathol* 1999;21(5):307-312.
73. Woolner LB, McConahey WM, Beahrs OH: Invasive fibrous thyroiditis (Riedel's struma). *J Clin Endocrinol Metab* 1957;17:201-220.
74. Harigopal M, Sahoo S, Recant WM, DeMay RM: Fine-needle aspiration of Riedel's disease: Report of a case and review of the literature. *Diagn Cytopathol* 2004;30(3):193-197.
75. Gharib H, Goellner JR: Diagnosis of amyloidosis by fine-needle aspiration biopsy of the thyroid [Letter]. *N Engl J Med* 1981;305:586.
76. Hamed G, Heffess CS, Shmookler BM, Wenig BM: Amyloid goiter: A clinicopathologic study of 14 cases and review of the literature. *Am J Clin Pathol* 1995;306-312.
77. Keyhani-Rofagha S, Kooner DS, Landas SK, Keyhani M: Black thyroid: A pitfall for aspiration cytology. *Diagn Cytopathol* 1991;7:640-643.
78. Wajda KJ, Wilson MS, Lucas J, Marsh WL: Fine needle aspiration cytologic findings in the black thyroid syndrome. *Acta Cytol* 1988;32:862-865.
79. Centeno BA, Szyfelbein WM, Daniels GH, Vickery AL: Fine-needle aspiration biopsy of the thyroid gland in patients with prior Graves' disease treated with radioactive iodine: Morphologic findings and potential pitfalls. *Acta Cytol* 1996;40:1189-1197.
80. Granter SR, Cibas ES: Cytologic findings in thyroid nodules after 131iodine treatment of hyperthyroidism. *Am J Clin Pathol* 1997;107:20-25.

81. Smejkal V, Smejkalova E, Rosa M, et al.: Cytologic changes simulating malignancy in thyrotoxic goiters treated with carbimazole. *Acta Cytol* 1985;29:173-178.
82. Mazzaferri EL: NCCN thyroid carcinoma practice guidelines. *Oncology* 1999;13:391-442.
83. Baloch ZW, Fleisher S, LiVolsi VA, Gupta PK: Diagnosis of "follicular neoplasm": A gray zone in thyroid fine-needle aspiration cytology. *Diagn Cytopathol* 2002;26(1):41-44.
84. Civantos F, Albores-Saavedra J, Nadji M, Morales AR: Clear cell variant of thyroid carcinoma. *Am J Surg Pathol* 1984;8:187-192.
85. Gonzalez JL, Wang HH, Ducatman BS: Fine needle aspiration of Hürthle cell lesions: A cytomorphologic approach to diagnosis. *Am J Clin Pathol* 1993;100:231-235.
86. Giordadze T, Rossi ED, Fadda G, et al.: Does the fine-needle aspiration diagnosis of "Hürthle-cell neoplasm/follicular neoplasm with oncocytic features" denote increased risk of malignancy? *Diagn Cytopathol* 2004;31(5):307-312.
87. Pu RT, Yang J, Wasserman PG, et al.: Does Hurthle cell lesion/neoplasm predict malignancy more than follicular lesion/neoplasm on thyroid fine-needle aspiration? *Diagn Cytopathol* 2006;34(5):330-334.
88. Silver SA, Busseniers AE: Cytologic criteria for differentiating neoplastic from nonneoplastic Hürthle cell lesions by fine needle aspiration (FNA) [Abstract]. *Mod Pathol* 1995;8:44A.
89. Renshaw AA: Hurthle cell carcinoma is a better gold standard than Hurthle cell neoplasm for fine-needle aspiration of the thyroid: defining more consistent and specific cytologic criteria. *Cancer* 2002;96(5):261-266.
90. LiVolsi VA: Surgical pathology of the thyroid. In Bennington JL (ed): *Major Problems in Pathology*, vol 22. Philadelphia, WB Saunders, 1990.
91. Rosai J, Carcangiu ML, DeLellis RA: Tumors of the thyroid gland. In DeLellis RA (ed): *Atlas of Tumor Pathology*, 3rd series, Fascicle 5. Washington, DC: Armed Forces Institute of Pathology, 1992.
92. Papotti M, Manazza AD, Chiarle R, Bussolati G: Confocal microscope analysis and tridimensional reconstruction of papillary thyroid carcinoma nuclei. *Virchows Arch* 2004;444(4):350-355.
93. Schlumberger M, Filetti S, Hay ID: Nontoxic goiter and thyroid neoplasia. In Larsen PR, Kronenberg HM, Melmed S, et al., (eds): *Williams' Textbook of Endocrinology*, 10th ed. Philadelphia, Saunders, 2002.
94. Odashiro DN, Nguyen GK: Diffuse sclerosing variant papillary carcinoma of the thyroid: Report of four cases with fine-needle aspirations. *Diagn Cytopathol* 2006;34(3):247-249.
95. Tabbara SO, Acoury N, Sidawy MK: Multinucleated giant cells in thyroid neoplasms A cytologic, histologic and immunohistochemical study. *Acta Cytol* 1996;40(6):1184-1188.
96. Ellison E, Lapuerta P, Martin SE: Psammoma bodies in fine-needle aspirates of the thyroid: Predictive value for papillary carcinoma. *Cancer* 1998;84:169-175.
97. Muller N, Cooperberg PL, Suen KC, Thorson SC: Needle aspiration biopsy in cystic papillary carcinoma of the thyroid. *Am J Roentgenol* 1985;144:251-253.
98. Renshaw AA: "Histiocytoid" cells in fine-needle aspirations of papillary carcinoma of the thyroid: Frequency and significance of an under-recognized cytologic pattern. *Cancer* 2002;96(4):240-243.
99. Renshaw AA: Focal features of papillary carcinoma of the thyroid in fine-needle aspiration material are strongly associated with papillary carcinoma at resection. *Am J Clin Pathol* 2002;118(2):208-210.
100. Ellison E, Lapuerta P, Martin SE: Psammoma bodies in fine-needle aspirates of the thyroid: Predictive value for papillary carcinoma. *Cancer* 1998;84(3):169-175.
101. Goellner JR, Carney JA: Cytologic features of fine needle aspirates of hyalinizing trabecular adenoma of the thyroid. *Am J Clin Pathol* 1989;91:115-119.
102. Kuma S, Hirokawa M, Miyauchi A, et al.: Cytologic features of hyalinizing trabecular adenoma of the thyroid. *Acta Cytol* 2003;47(3):399-404.
103. Cheung CC, Boerner SL, MacMillan CM, et al.: Hyalinizing trabecular tumor of the thyroid: A variant of papillary carcinoma proved by molecular genetics. *Am J Surg Pathol* 2000;24:1623-1626.
104. Papotti M, Volante M, Giuliano A, et al.: RET/PTC activation in hyalinizing trabecular tumors of the thyroid. *Am J Surg Pathol* 2000;24:1615-1621.
105. LiVolsi VA: Hyalinizing trabecular tumor of the thyroid: Adenoma, carcinoma, or neoplasm of uncertain malignant potential? [Editorial]. *Am J Surg Pathol* 2000;24:1683-1684.
106. Logani S, Gupta PK, LiVolsi VA, et al.: Thyroid nodules with FNA cytology suspicious for follicular variant of papillary thyroid carcinoma: follow-up and management. *Diagn Cytopathol* 2000;23(6):380-385.
107. Pietribiasi F, Sapino A, Papotti M, Bussolati G: Cytologic features of poorly differentiated "insular" carcinoma of the thyroid, as revealed by fine-needle aspiration biopsy. *Am J Clin Pathol* 1990;94:687-692.
108. Sironi M, Collini P, Cantaboni A: Fine needle aspiration cytology of insular thyroid carcinoma: A report of four cases. *Acta Cytol* 1992;36:435-439.
109. Guiter GE, Auger M, Ali SZ, et al.: Cytopathology of insular carcinoma of the thyroid. *Cancer (Cancer Cytopathol)* 1999;87:196-202.
110. Nguyen GK, Akin M-RM: Cytopathology of insular carcinoma of the thyroid. *Diagn Cytopathol* 2001;25:325-330.
111. Layfield LJ, Gopez EV: Insular carcinoma of the thyroid: report of a case with intact insulae and microfollicular structures. *Diagn Cytopathol* 2000;23:409-413.
112. Carcangiu ML, Steeper T, Zampi G, Rosai J: Anaplastic thyroid carcinoma: A study of 70 cases. *Am J Clin Pathol* 1985;83:135-158.
113. Venkatesh YS, Ordonez NG, Schultz PN, et al.: Anaplastic carcinoma of the thyroid: A clinicopathologic study of 121 cases. *Cancer* 1990;66:321-330.
114. Nonaka D, Tang Y, Chiriboga L, et al.: Diagnostic utility of thyroid transcription factors Pax8 and TTF-2 (FoxE1) in thyroid epithelial neoplasms. *Mod Pathol* 2008;21(2):192-200.
115. Us-Krasovec M, Golouh R, Auersperg M, et al.: Anaplastic thyroid carcinoma in fine-needle aspirates. *Acta Cytol* 1996;40:953-958.
116. Us-Krasovec M, Auersperg M, Bergant D, et al.: Medullary carcinoma of the thyroid gland: Diagnostic cytopathologic characteristics. *Pathologica* 1998;90:5-13.
117. Derringer GA, Thompson LDR, Frommelt RA, et al.: Malignant lymphoma of the thyroid gland: A clinicopathologic study of 108 cases. *Am J Surg Pathol* 2000;24:623-639.
118. Schmid KW, Hittmair A, Ofner C, et al.: Metastatic tumors in fine needle aspiration biopsy of the thyroid. *Acta Cytol* 1991;35:722-724.
119. Smith SA, Gharib H, Goellner JR: Fine-needle aspiration: usefulness for diagnosis and management of metastatic carcinoma to the thyroid. *Arch Intern Med* 1987;147:311-312.
120. Papi G, Fadda G, Corsello SM, et al.: Metastases to the thyroid gland: Prevalence, clinicopathological aspects and prognosis: a 10-year experience. *Clin Endocrinol (Oxf)* 2007;66(4):565-571.
121. Tambouret R, Szyfelbein WM, Pitman MB: Ultrasound-guided fine-needle aspiration biopsy of the thyroid. *Cancer (Cancer Cytopathol)* 1999;87:299-305.
122. Winkler B, Gooding GA, Montgomery CK, et al.: Immunoperoxidase confirmation of parathyroid origin of ultrasound-guided fine needle aspirates of the parathyroid glands. *Acta Cytol* 1987;31:40-44.

Salivary Gland

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Adenocarcinoma, Not Otherwise Specified

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MISCELLANEOUS

RATIONALE, INDICATIONS, AND TECHNICAL CONSIDERATIONS

Any unexplained salivary gland mass is an indication for fine-needle aspiration (FNA). FNA is the preferred biopsy method because incisional biopsy is associated with an increased risk of infection and potential contamination of surgical planes. FNA, in contrast, is a cost-effective technique¹⁻¹⁰ that poses minimal risk to the patient. The few contraindications to FNA are a bleeding disorder (which might result in a hematoma) and acute sialadenitis (associated with aspiration-related pain); obscuring blood or inflammatory cells, in these settings, can limit diagnostic interpretation.

Clinical and imaging findings cannot always establish the origin of tumors in the head and neck region. Thus, a primary goal of FNA is to distinguish salivary gland lesions from nonsalivary head and neck masses, especially those of lymph node origin, but also soft tissue lesions, and skin and skin adnexal masses. Because the normal parotid gland contains more than 20 intraparotid

and periparotid lymph nodes,¹¹ primary lymphomas and metastatic tumors mimicking salivary gland neoplasms are commonly seen.

Some authors have argued that FNA is superfluous because mass lesions in the head and neck always require excision.^{12,13} Such an approach would result in the unnecessary excision of a number of lesions (up to one third of salivary gland masses) that in fact do not require surgery. Some patients have a non-neoplastic lesion (e.g., chronic sialadenitis, lymphoepithelial cysts, granulomatous diseases); others have a benign neoplasm but, because they are poor surgical candidates, knowledge of its benignity provides reassurance that surgery can be avoided; and still others have a malignancy for which surgical excision is not appropriate (e.g., lymphoproliferative disease, metastasis). Even when surgery is indicated, FNA guides preoperative strategy (e.g., partial or total parotidectomy, facial nerve resection, neck dissection), providing reassurance to both the patient and surgeon.^{2,7,12} Surgical management is often influenced by cytologic findings. High-grade malignancies are treated

more aggressively than low-grade malignancies and benign neoplasms. In the superficial parotid gland, the most common site of salivary gland neoplasms, benign tumors and low-grade malignancies are treated with superficial parotidectomy alone, whereas high-grade carcinomas are treated with total parotidectomy possibly requiring facial nerve sacrifice. Furthermore, lymph node neck dissection and neoadjuvant therapy are often indicated for high-grade tumors.

Aspirations are performed using a 25- or 23-gauge needle, small enough to reduce the risk of tissue trauma but large enough to obtain an adequately cellular sample. Evaluation of direct smears at the time of the procedure can guide the number of passes required by assessing adequacy and also facilitate appropriate triage of the specimen, like allocation of material for flow cytometry (in the case of lymphoid lesions) or cell block preparations (in the case of diagnostically challenging lesions such as oncocytic, spindle cell, and clear cell tumors).

Although FNA was first described in the 1930s,¹⁴⁻¹⁶ salivary gland FNA is a relatively new discipline, having gained wide acceptance only in the last 35 years.^{1,2,5-8,17-27} In the most recent studies, sensitivity and specificity for neoplasia is over 90%. The distinction between benign and malignant neoplasms has a sensitivity of 80% to 90% and a specificity of over 90%.

Criteria for assessing adequacy have yet to be established. False-negative diagnoses are principally a result of inadequate sampling of the lesion,²⁸ most frequently those that are cystic. Cystic neoplasms (e.g., Warthin tumor, low-grade mucoepidermoid carcinoma, metastatic squamous cell carcinoma) are the most likely causes of false-negative diagnoses. Cysts frequently yield nonspecific fluid not representative of the underlying lesion. When a cystic salivary gland lesion is aspirated, any residual mass should be resampled after fluid is withdrawn. Surgical excision is indicated if the cyst does not resolve by aspiration or if it recurs.

False-negatives resulting from an interpretation error are most common with low-grade mucoepidermoid carcinoma, adenoid cystic carcinoma, and non-Hodgkin lymphoma. False-positive diagnoses are seen with cystic lesions, particularly Warthin tumor, which sometimes contains atypical squamous cells, and pleomorphic adenomas, in which nuclear atypia or stromal spheres result in an incorrect diagnosis of carcinoma ex pleomorphic adenoma or adenoid cystic carcinoma, respectively. Multiple passes help to minimize sampling and interpretative errors resulting from variation in cytologic atypia and cellular constituents within a tumor.

Complications are infrequent. Tumor seeding in the needle tract is extremely rare.^{12,29} The most common complications are bleeding, infection, and facial nerve pain. In some cases, FNA leads to partial, or rarely, complete infarction of the neoplasm.³⁰⁻³² Particularly susceptible neoplasms are oncocytoma, Warthin tumor,

and acinic cell carcinoma.^{12,33} When using a 25-gauge needle, significant infarction or hemorrhage is seen in up to 10% of cases, but such changes rarely hinder histologic diagnosis.³³

Both Romanowsky-type and Papanicolaou stains are important because many neoplasms contain a combination of epithelial and stromal components. Air-dried Romanowsky-stained smears highlight diagnostically useful features of the stromal component that are poorly visualized in alcohol-fixed preparations of lesions, such as pleomorphic adenoma, basal cell tumors, and adenoid cystic carcinoma. Romanowsky stains also aid in the evaluation of lymphoid lesions. Papanicolaou-stained smears are especially useful for evaluating nuclear features and cytoplasmic differentiation.

Either smears or liquid-based preparations can be used,³⁴⁻³⁶ but conventional smears are preferred. With liquid-based preparations, extracellular constituents are less prominent, cellular shrinkage is greater, and tissue fragmentation is more pronounced, with possible decreased sensitivity and specificity.³⁴ Although the role of immunohistochemical stains is limited, a cell block is valuable for histochemical stains such as periodic acid-Schiff (PAS) and mucicarmine. Cell block preparations also better demonstrate architectural patterns and some cellular features, particularly serous acinar differentiation.³⁷

DIAGNOSTIC OVERVIEW

Precise classification of salivary gland neoplasms by FNA is possible for many of the commonly encountered lesions, but remains problematic for a number of the less common entities. Salivary gland FNA, in fact, poses a number of challenges to the cytopathologist. First, there are more than 35 salivary gland tumors of epithelial type,^{38,39} many of which are rare, placing familiarity with them out of reach for most practitioners. Second, most salivary gland malignancies are low-grade, displaying few overt cytologic features of malignancy. Third, the less common, high-grade malignancies are readily recognizable as malignant, but they are difficult to distinguish from one another. Fourth, some benign tumors (e.g., basal cell adenoma) have a malignant counterpart (e.g., basal cell adenocarcinoma) that is morphologically identical except that there is an infiltrative growth pattern, something that cannot be assessed cytologically. Finally, many different salivary gland tumors share similar cellular constituents; it is the architectural relationship and relative abundance of these constituents that ultimately determines the tumor type. With some tumors (such as pleomorphic adenoma), there is great variability not just within a given tumor but from one tumor to the next.

Fortunately for the cytopathologist, there are two mitigating factors. First, the two most common neoplasms, pleomorphic adenoma and Warthin tumor, which together comprise over 80% of salivary gland tumors, have distinctive cytomorphologic features and are readily identified. Second, conservative excision is used for both benign tumors and low-grade malignancies. In contrast, radical surgical approaches with combined modality therapy are reserved for high-grade malignancies. Thus, although a specific diagnosis may not be feasible, low-grade neoplasms usually can be distinguished from high-grade ones, and an appropriate differential diagnosis is sufficient for clinical management. This chapter provides a diagnostic approach and suggested reporting terminology for such cases.

The large group of so-called “basaloid neoplasms” are a special case, however. The basaloid neoplasms encompass the entire spectrum of biologic behavior, from benign neoplasms, through low-grade malignancies, to the aggressive solid variant of adenoid cystic carcinoma. A precise cytologic diagnosis is not possible with many FNAs that show basaloid cells only. A frozen section is often necessary to guide appropriate surgical management. This and other diagnostic dilemmas are listed herein. Suggested diagnostic approaches are discussed in detail in the remainder of the text.

Diagnostic dilemmas in salivary gland fine-needle aspiration:

- basaloid neoplasms (especially basal cell adenoma and the solid variant of adenoid cystic carcinoma)
- oncocytic lesions (especially oncocytoma and acinic cell carcinoma)
- mucus-containing cysts (especially mucoepidermoid carcinoma, mucocele, and mucinous metaplasia)
- high-grade carcinomas (including mucoepidermoid carcinoma, salivary duct carcinoma, and carcinoma ex pleomorphic adenoma)
- clear cell neoplasms
- spindle cell lesions (especially myoepithelioma and schwannoma)

Clinical information is often helpful, such as knowledge of a previous malignancy. Aggressive signs, symptoms, and imaging findings, like rapid growth, pain (suggestive of neural invasion), and infiltrative growth on radiological studies, are indicative of malignancy. Most salivary gland neoplasms are firm and painless, although Warthin tumor has a characteristically doughy consistency.

The presence of cystic change and bilateral nature can also help narrow down diagnostic possibilities.

Tumors that are often cystic:

- Warthin tumor
- mucoepidermoid carcinoma
- acinic cell carcinoma

Lesions that are sometimes bilateral:

- sialadenitis
- amyloidosis
- lymphoepithelial cyst
- Warthin tumor
- acinic cell carcinoma
- lymphoma

The epidemiological features of salivary gland neoplasms are also helpful. Most salivary gland neoplasms are more common in women, but Warthin tumor, salivary duct carcinoma, and metastatic squamous cell carcinoma occur more frequently in men. Whereas 68% to 85% of parotid gland tumors are benign, 80% to 90% of sublingual and minor salivary gland neoplasms are malignant.⁴⁰ Some tumors are almost site-specific (e.g., Warthin tumor in the parotid gland, polymorphous low-grade adenocarcinoma in the minor salivary glands).

Attention to the constituents of an aspirate is the key to the neoplastic, inflammatory, lymphoid, or cystic nature of a lesion. *Hypercellular specimens* are typical of neoplastic lesions. *Inflammatory cells* are prominent in sialadenitis and cystic lesions. *Stone fragments* are diagnostic of sialolithiasis. *Abundant lymphoid cells* can be seen in a variety of salivary gland lesions, not all of them exclusively lymphoid in nature.

Numerous lymphocytes are seen in:

- intraparotid or periparotid lymph node
- lymphoma
- lymphoepithelial cyst
- Warthin tumor
- mucoepidermoid carcinoma
- acinic cell carcinoma
- lymphoepithelial carcinoma

The presence and character of matrix material provides important diagnostic information. *Mucin* (pale magenta in Romanowsky preparations, translucent blue/purple on Papanicolaou smears) suggests a mucoepidermoid carcinoma, mucocele, retention cyst, or mucinous metaplasia. A *chondromyxoid matrix* is characteristic of pleomorphic adenoma and *stromal spheres* are typical of adenoid cystic carcinoma, but neither finding is entirely specific. Such findings, along with a more detailed impression of the cell type(s) seen (myoepithelial, duct-lining epithelial, basaloid, oncocytic, mucinous, squamous, acinic, sebaceous, and clear cells) enable one to refine the differential diagnosis.

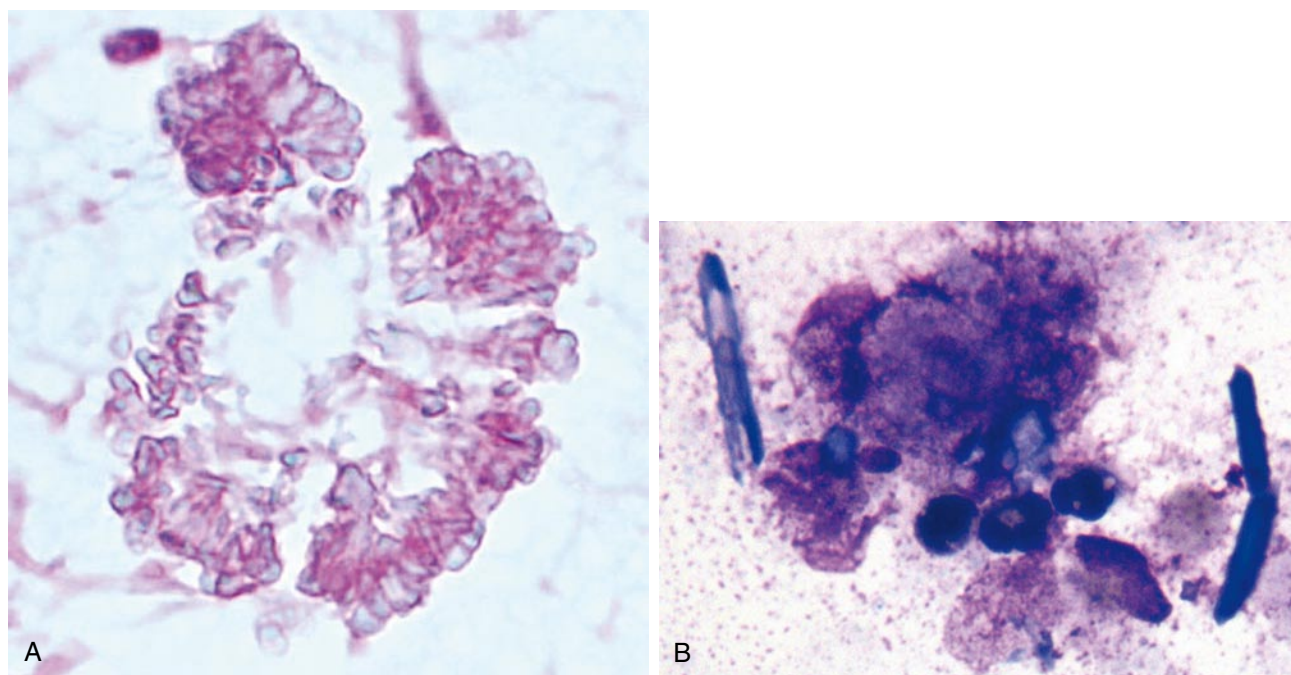


Figure 10.1 Salivary gland crystalloids. A, Tyrosine crystalloids are clustered in flower-like arrangements (hematoxylin-eosin [H & E] stain). (Courtesy of Dr. Celeste Powers, Virginia Commonwealth University). B, Amylase crystalloids, needle shaped or plate-like, are occasionally encountered in salivary gland fine-needle aspirations (FNAs), usually in association with inflammatory conditions (Romanowsky stain).

A variety of crystalloids are seen in the salivary glands.^{41,42} Importantly, none of them is specific for any particular salivary gland lesion or neoplasm. *Tyrosine crystalloids* are floret-shaped and often encountered in pleomorphic adenomas, but they can be seen in other lesions, both benign and malignant (Fig. 10.1A).⁴¹⁻⁴⁶ *Amylase crystalloids* (“nontyrosine crystalloids”) are polygonal, plate-like, or needle-shaped and are most often seen in benign, non-neoplastic conditions, especially infections and cysts⁴⁷⁻⁵⁰ (Fig. 10.1B).

THE NORMAL ASPIRATE

Some FNAs, often as a result of sampling error, show only normal salivary gland elements.

CYTOMORPHOLOGY OF NORMAL SALIVARY GLAND ELEMENTS:

- rounded clusters of serous or mucinous acinar cells
- flat sheets and tubules of ductal cells
- adipose tissue

Aspirates of normal salivary gland tissue are sparsely cellular, comprised of acinar cells, ductal cells, and admixed adipose tissue^{4,51} (Fig. 10.2A). Occasionally, naked acinar nuclei that mimic lymphocytes and

scattered myoepithelial cells are present. Acinar cells are usually arranged in cohesive, grape-like clusters. Ductal cells are smaller and less conspicuous, arranged as tubules or honeycomb-like flat sheets. The acinar cells are of serous type in the parotid gland, a mixture of serous and mucinous types in the submandibular gland, and predominantly mucinous in the minor salivary glands. The serous-type acinar cells (Fig. 10.2B) are evenly spaced, with basally placed nuclei. They are large, pyramidal cells with abundant foamy and granular, basophilic cytoplasm and small, eccentrically placed, round to oval nuclei with indistinct nucleoli. Normal (and neoplastic) acinar cells have characteristic coarse, basophilic, PAS-positive and diastase-resistant cytoplasmic zymogen granules. In contrast to the pyramidal serous cells, mucinous cells are columnar, with pale cytoplasm that indents a bland nucleus. Ductal cells come in several varieties. Intercalated duct cells are uniform, small, and cuboidal, with scant, dense cytoplasm and uniform nuclei; occasional large branching ductal fragments are present. Ductal cells derived from the larger striated ducts are oncocytic, whereas those from the collecting ducts are columnar and ciliated. Ductal cells sometimes exhibit mucinous or squamoid changes. Mature lymphocytes can also be seen, owing to the abundance of intraparotid and periparotid lymphoid tissue. A mixture of ductal cells, adipose tissue, and acinar cells distinguishes normal salivary gland tissue from acinic cell

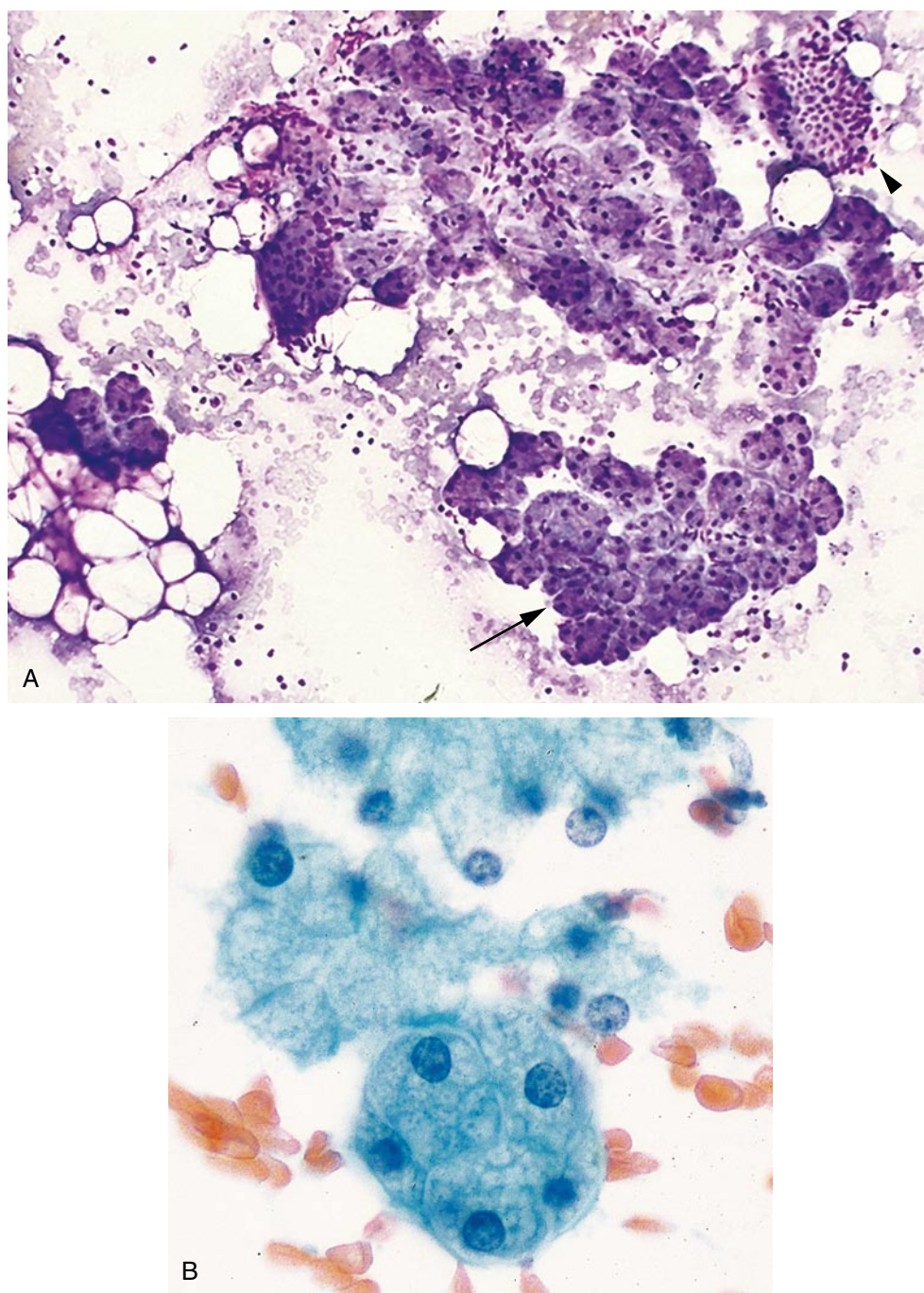


Figure 10.2 Normal salivary gland. A, Normal acinar cells are aggregated in grape-like bunches (arrow) with admixed adipose tissue and an occasional flat sheet of ductal cells (arrowhead; Romanowsky stain). B, Acinar cells have uniform, round nuclei and abundant vacuolated cytoplasm (Papanicolaou stain).

carcinoma, which usually consists of a monomorphic population of neoplastic acinar cells.

Up to 20% of salivary gland aspirates yield only normal tissue.^{5,51} An FNA that reveals only normal salivary gland elements warrants clinical correlation to exclude the possibility of sampling error. Other explanations for a normal-elements-only result include a prominent but normal salivary gland, sialadenosis, hamartoma, and lipoma.

NON-NEOPLASTIC CONDITIONS

Acute and Chronic Sialadenitis

Acute sialadenitis is rarely aspirated because it is usually diagnosed clinically as a postoperative complication, viral or fungal infection, or secondary bacterial infection resulting from an obstruction such as sialolithiasis⁵²⁻⁵⁵ (Fig. 10.3). There is usually no discrete mass, and most cases affect the parotid gland.

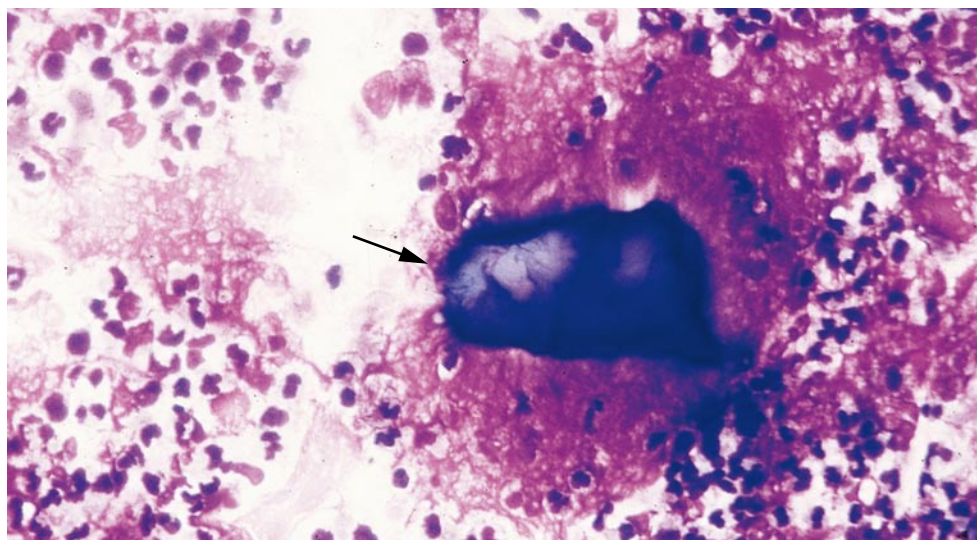


Figure 10.3 Sialolithiasis with acute sialadenitis. Stone fragments (arrow) are blue, irregularly shaped, jagged structures of varying sizes that are diagnostic of sialolithiasis. The presence of numerous neutrophils signifies an accompanying acute sialadenitis (Romanowsky stain).

CYTOMORPHOLOGY OF ACUTE SIALADENITIS:

- neutrophils, necrotic debris
- stone fragments (if sialolithiasis also present)
- scant ductal cells

Fine needle aspirates of acute sialadenitis show abundant neutrophils, necrotic cells, and fibrin.⁵² Small groups of ductal cells, some with reactive atypia, are present. A malignant neoplasm is excluded based on the hypocellularity and limited atypia. When an infectious etiology is suspected, a portion of the material should be sent for a microbiologic work-up. If there is a clinical suspicion of a neoplastic process, reaspiration after treatment and resolution of the acute infection might be helpful.

As with acute sialadenitis, FNA of *chronic sialadenitis* can be associated with pain. Chronic sialadenitis is more likely to present as a clinically discrete mass, often in the

submandibular gland. Common causes include sialolithiasis and radiation therapy for head and neck cancer (usually squamous cell carcinoma).

CYTOMORPHOLOGY OF CHRONIC SIALADENITIS:

- scant cellularity
- small sheets and tubules of basaloid ductal cells with sharp borders
- paucity of acinar elements
- blood, proteinaceous debris, mature lymphocytes
- fragments of fibrous tissue

Aspirates of chronic sialadenitis are sparsely cellular. Clusters of small, basaloid ductal cells with sharp borders are admixed with blood, proteinaceous debris, mature lymphocytes, and small amounts of fibrous tissue (Fig. 10.4). Acinar cells are sparse or absent.⁵² Sialolithiasis can be diagnosed when stone fragments are identified

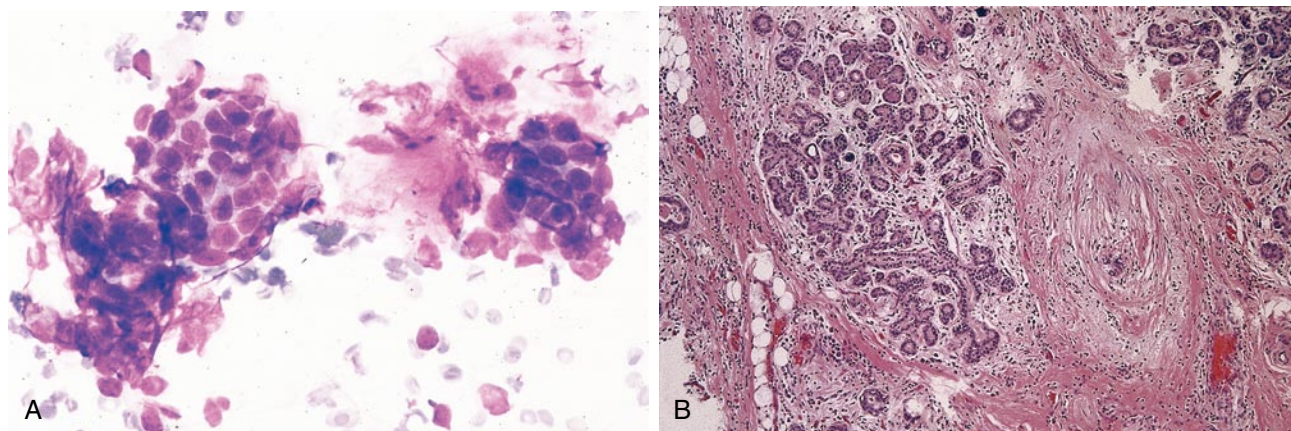


Figure 10.4 Chronic sialadenitis. A, Sparsely cellular smears show small cohesive clusters of basaloid ductal cells that can be mistaken for the cells of a basaloid neoplasm (Romanowsky stain). B, The corresponding histologic specimen shows marked fibrosis, chronic inflammation, acinar atrophy, and residual ductal elements (hematoxylin-eosin [H & E] stain).

(see Fig. 10.3). Atypical squamous metaplasia, mucinous metaplasia, radiation atypia, abundant histiocytes, extracellular mucin, crystals, and (rarely) psammoma bodies may be present.⁵⁶ *Chronic sclerosing sialadenitis (Kuttner tumor)* is a form of chronic sialadenitis affecting the submandibular gland and presenting clinically as a firm mass that can be mistaken for a neoplastic process.^{38,57-59} The morphologic findings are non-specific and similar to those of a conventional chronic sialadenitis. In some cases, lymphoid cells are abundant and lymphoma must be excluded.⁵⁹

DIFFERENTIAL DIAGNOSIS OF CHRONIC SIALADENITIS

- normal salivary gland
- lymphoepithelial sialadenitis (LESA)
- Warthin tumor
- basaloid neoplasms
- mucoepidermoid carcinoma
- squamous cell carcinoma

Normal salivary gland has a greater proportion of acinar-to-ductal cells than chronic sialadenitis. Chronic sialadenitis is distinguished from lymphoepithelial sialadenitis (LESA) by the absence of lymphoepithelial islands and germinal center fragments. Unlike Warthin tumor, chronic sialadenitis lacks cohesive groups of oncocytes. The basaloid ductal cells of chronic sialadenitis can resemble the cells of basaloid neoplasms but are less numerous and arranged in smaller groups than those of neoplasms. The intermediate cells of a mucoepidermoid carcinoma resemble the basaloid ductal cells of chronic sialadenitis, but mature squamous cells and mucus cells are absent. Chronic sialadenitis is separated from a squamous cell carcinoma by virtue of its scant cellularity; the absence of marked atypia, mitotic activity, and necrotic tumor cells; and the paucity of isolated epithelial cells.

Granulomatous Sialadenitis

Granulomatous sialadenitis can be caused by infection (fungi, mycobacteria, toxoplasmosis, and cat scratch disease), sarcoidosis, cyst rupture, and rarely neoplasia (Hodgkin lymphoma, T-cell lymphoma, or metastatic carcinoma).⁶⁰⁻⁶² Aspirates are characterized by a variable background of granular, necrotic cell debris and inflammation, together with aggregates of epithelioid histiocytes, frequently with multinucleated forms. Epithelioid histiocytes have abundant eosinophilic cytoplasm and cytologically bland, elongated, folded nuclei with indistinct nucleoli. Asteroid bodies, Schaumann bodies, and calcium oxalate crystals can be present in sarcoidosis, but these findings are not specific, and an infectious etiology must be excluded by special stains or microbiologic cultures.⁶³

Sialadenosis

Sialadenosis is a non-neoplastic, noninflammatory enlargement of the salivary gland that more commonly affects the parotid gland and is often bilateral.^{52,64,65} It results from acinar cell hypertrophy, and has been associated with a wide range of etiologies, including endocrine abnormalities like diabetes mellitus, nutritional deficiencies, alcoholism, cirrhosis, and certain drugs, especially antihypertensives.^{38,53,64}

Aspirates of sialadenosis appear normal except that the constituent acinar cells are significantly larger than normal acinar cells, and inflammatory cells tend to be absent. Normal acinar cells are approximately 50 µm in diameter, whereas acinar cells of sialadenosis can measure up to 100 µm.^{38,52,53,64} In practice, these size differences are difficult to assess. Clinical correlation to exclude a discrete mass or neoplastic lesion is important before making a diagnosis of sialadenosis.

Lymphoepithelial Sialadenitis

This lesion has been known by a variety of names, including Mikulicz disease, benign lymphoepithelial lesion, and myoepithelial sialadenitis (MESA).^{66,67} Resulting in part from the discovery that the cells comprising the lymphoepithelial islands (formerly called epimyoeplithelial islands) of this disorder are almost entirely epithelial, the term *lymphoepithelial sialadenitis* has emerged as the preferred terminology.^{66,68} LESA most frequently affects women and presents as diffuse, often bilateral, enlargement of the parotid gland and the submandibular gland in a minority of cases. Minor salivary glands show chronic inflammatory changes related to LESA, but lack the lymphoepithelial islands. LESA is believed to be an autoimmune disorder seen in virtually all patients with Sjögren syndrome; however, approximately 50% of patients with LESA do not have Sjögren syndrome. They may have some other connective tissue disorder or no disease whatever.⁶⁸

CYTOMORPHOLOGY OF LYMPHOEPITHELIAL SIALADENITIS:

- cellular aspirate
- mixed population of lymphocytes, plasma cells, tingible-body macrophages
- germinal center fragments
- lymphoepithelial islands

Aspirates are cellular and show a mixed population of mature lymphocytes, plasma cells, tingible-body macrophages, germinal center fragments, and characteristic lymphoepithelial islands: large, cohesive sheets of pale, overlapping, ductal-type cells infiltrated by lymphocytes (Fig. 10.5). The ductal cells often exhibit reactive and squamous metaplastic changes. Acinar cells are rarely present.

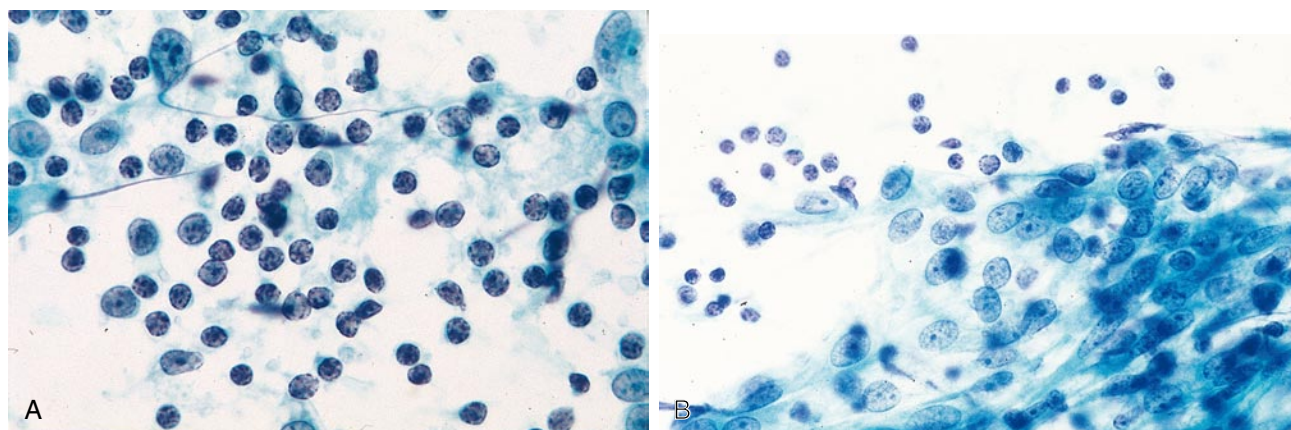


Figure 10.5 Lymphoepithelial sialadenitis (LESA). A, Lymphoid cells, predominantly small lymphocytes, are usually abundant (Papanicolaou stain). B, The characteristic lymphoepithelial islands are composed of reactive epithelial cells admixed with lymphocytes (Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF LYMPHOEPITHELIAL SIALADENITIS

- chronic sialadenitis
- simple lymphoepithelial cyst
- human immunodeficiency virus (HIV)-associated cystic lymphoepithelial lesions
- Warthin tumor
- extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT)

Chronic sialadenitis tends to be sparsely cellular, with fewer lymphocytes and germinal center fragments, and lacks the characteristic lymphoepithelial islands of LESA. Simple lymphoepithelial cysts and human immunodeficiency virus (HIV)-associated cystic lymphoepithelial lesions are cytologically similar to the cystic form of LESA and are discussed further in the next section. Warthin tumor is distinguished from LESA because the former contains oncocytic epithelium. Perhaps most importantly, mucosa-associated lymphoid tissue (MALT) lymphoma should be suspected if large numbers of monocytoid B-cells are encountered. Ancillary studies for clonality such as flow cytometry or immunocytochemistry should be employed liberally.

Non-Neoplastic Cysts

Cystic lesions account for approximately 5% of salivary gland FNAs. A wide variety of non-neoplastic and neoplastic lesions can be cystic.⁶⁹ Non-neoplastic cysts, both congenital and acquired, occur either adjacent to or within the salivary glands.^{38,52,70} Diagnostically, these cysts can be broadly categorized into squamous-lined cysts and mucus-containing cysts.

Squamous-Lined Cysts

Squamous-lined cysts include *congenital cysts*, encompassing dermoid and branchial cleft cysts. Also in this category are the sporadic *simple lymphoepithelial cysts*, usually found within the parotid glands of middle-aged men.⁶⁸ Simple lymphoepithelial cysts are not related to HIV infection or Sjögren syndrome. Typically unilateral and solitary, they probably arise from either entrapped salivary duct tissue within intraparotid lymph nodes or from branchial cleft remnants.⁶⁸ In contrast, *HIV-associated cystic lymphoepithelial lesions* are usually multiple and often bilateral. Squamous-lined cysts yield clear to turbid yellow-brown fluid.

CYTOMORPHOLOGY OF SQUAMOUS-LINED CYSTS:

- cellular aspirate
- histiocytes
- keratin debris and anucleate squames
- small clusters of squamous (or columnar) cells
- mixed population of lymphocytes
- with or without germinal center fragments with tingible-body macrophages
- absence of lymphoepithelial islands (except in HIV-associated cases and cystic LESA)

The aspirate findings are non-specific, showing a mixed population of lymphocytes, often with germinal center fragments, tingible-body macrophages, keratin debris, squamous or columnar cyst lining cells, histiocytes, and proteinaceous debris^{71,72} (Fig. 10.6A). Within this group of lesions, finding epithelial cell clusters with interspersed lymphocytes (called *lymphoepithelial islands*) is indicative of either a cystic LESA or an HIV-associated cystic lymphoepithelial lesion. Clinical and radiographic correlation is helpful in this distinction.

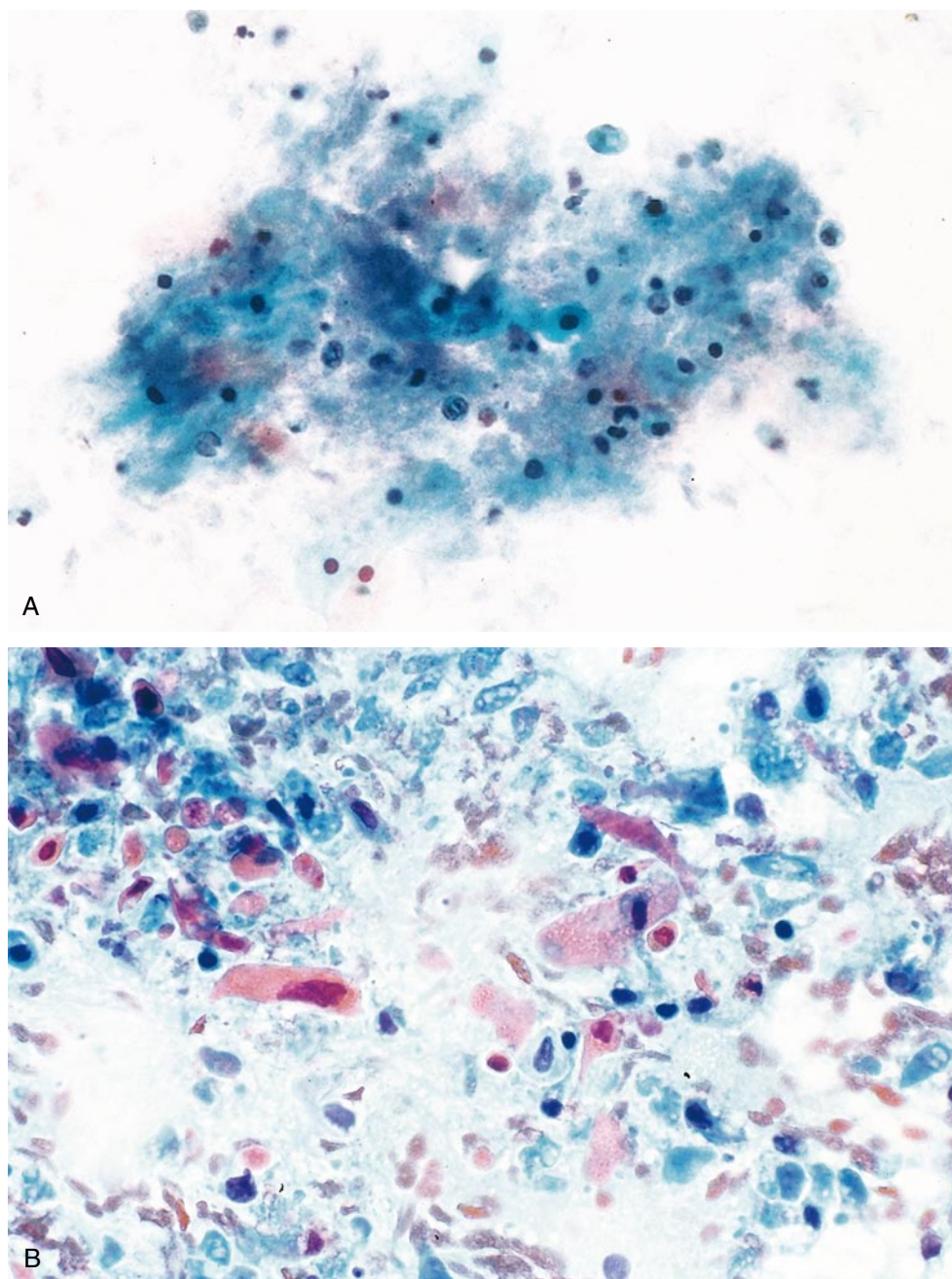


Figure 10.6 Squamous epithelial-lined cyst compared to squamous cell carcinoma. *A*, In a benign squamous-lined epithelial cyst, degenerated and anucleate squamous cells with minimal nuclear atypia are present. These findings are non-specific (Papanicolaou stain). *B*, In contrast, keratinizing squamous cell carcinoma has marked nuclear atypia, bizarre cell shapes, and necrosis (Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF SQUAMOUS-LINED CYSTS:

- pure lymphoid lesions (intraparotid lymph node, lymphoma)
- cystic salivary gland neoplasms (especially Warthin tumor, acinic cell carcinoma, and mucoepidermoid carcinoma)
- cystic metastatic squamous cell carcinoma

The presence of an epithelial component distinguishes these cysts from a lymph node. Lymphoid elements do not help in the distinction from a cystic salivary gland neoplasm because some neoplasms have a prominent lymphoid infiltrate. A lymphoma, especially of MALT type, can be excluded using flow cytometry, which demonstrates a polyclonal population of lymphoid cells in these benign cystic lesions. Distinction from a Warthin tumor can be especially difficult, because the oncocytes of a Warthin tumor can

show extensive squamoid differentiation. Identification of a salivary gland neoplasm rests on the recognition of the characteristic cell type(s) associated with that tumor (e.g., oncocytes, acinar cells, mucus, intermediate, and squamous cells). Most importantly, a cystic metastatic squamous cell carcinoma must also be considered. Although squamous-lined non-neoplastic cysts can exhibit reactive atypia, metastatic squamous cell carcinoma (Fig. 10.6B) contains necrotic cells, mitotic activity (including atypical mitoses), and more severe nuclear atypia. Nevertheless, a cystic well-differentiated squamous cell carcinoma is occasionally difficult to distinguish from a benign squamous-lined cyst. Beware of diagnosing a nonmidline developmental cyst in an older adult. Such aspirates should be thoroughly screened to exclude malignant features. Even in the absence of identifiable malignancy, it is prudent to report benign-appearing squamous-lined cysts descriptively and include the differential diagnosis of a developmental and lymphoepithelial cyst. An accompanying explanatory note can emphasize the need for clinical correlation to exclude a more significant lesion and a recommendation that any persistent mass be excised to exclude malignancy. The tonsil is often the primary site for a squamous cell carcinoma presenting as a cystic lesion. Because tonsillar squamous cell carcinomas are frequently associated with human papillomavirus (HPV) infection, HPV testing of these aspirates can be helpful both in confirming the diagnosis and in identifying a likely primary site.^{73,74}

Mucin-Containing Cysts

The umbrella term *mucin-containing cysts* refers to a heterogeneous group of lesions that include a malignant neoplasm (mucoepidermoid carcinoma), inflammatory conditions (chronic sialadenitis with mucinous metaplasia), and acquired cysts. Acquired cysts, comprising the mucocele and retention cyst, occur more commonly in the submandibular and sublingual glands than the parotid.^{38,70} *Mucocele*s are pseudocysts because they lack an epithelial lining, whereas *retention cysts* are lined by squamous, columnar, or oncocytic epithelium. Both result from obstruction, usually as a result of stones.

CYTOMORPHOLOGY OF MUCIN-CONTAINING CYSTS:

- sparsely cellular
- extracellular mucin
- histiocytes
- amylase crystalloids (non-specific)
- scattered inflammatory cells

Aspirates are sparsely cellular and comprised of histiocytes, some with intracellular mucin (“muciphages”), granular debris, extracellular mucin, amylase crystalloids, and inflammatory cells (Fig. 10.7). Occasional metaplastic epithelial cells and normal salivary gland cells are also seen.

DIFFERENTIAL DIAGNOSIS OF MUCIN-CONTAINING CYSTS

- mucocele
- retention cyst
- chronic sialadenitis with mucinous metaplasia of ducts
- mucoepidermoid carcinoma

Chronic sialadenitis with mucinous metaplasia of ducts has a similar appearance to the acquired cysts; ciliated columnar cells suggest the diagnosis. The nonspecific constellation of findings described herein is also seen in some *low-grade mucoepidermoid carcinomas*, which, like mucoceles and retention cysts, contain muciphages. The following features, typical of a low-grade mucoepidermoid carcinoma, can help distinguish it from one of its benign mimics: a residual mass after aspiration; greater cellularity; more severe cytologic atypia; and at least an occasional cluster of intermediate and epidermoid cells.

Whenever a specimen contains extracellular mucin but minimal (or even absent) cellular atypia, an “atypical” interpretation is desirable nevertheless. Mucoepidermoid carcinoma can be mentioned in the differential diagnosis, with an educational note. This diagnostic approach helps avoid a false-negative interpretation in the case of a low-grade mucoepidermoid carcinoma.

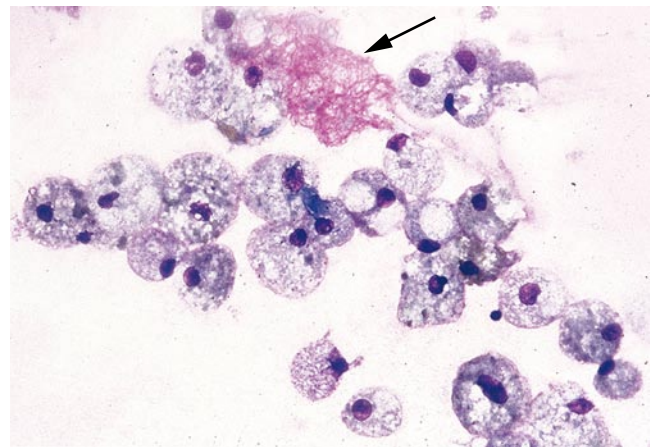


Figure 10.7 Mucus-containing cyst. The combination of histiocytes and magenta-colored extracellular mucin (arrow) is a nonspecific finding. This example proved to be a retention cyst, but identical findings can be seen in a low-grade mucoepidermoid carcinoma (MEC; Romanowsky stain).

Sample report of a mucin-containing cyst:**ATYPICAL**

Histiocytes (and epithelial elements) in a background of abundant mucin (see Note).

NOTE: The differential diagnosis includes a mucocele, mucus retention cyst, chronic sialadenitis with mucinous metaplasia, and a mucoepidermoid carcinoma. If the lesion persists or recurs, excision should be considered.

Amyloidosis

Amyloidosis is a rare, often bilateral cause of focal or diffuse salivary gland enlargement.⁷⁵⁻⁷⁷ Variable amounts of acellular, eosinophilic extracellular material stains pale red with the Congo red stain, and exhibits a characteristic apple-green birefringence under polarized light. The remainder of the smear is often hypocellular, with scant or absent acinar cells and scattered groups of ductal cells such as are seen in chronic sialadenitis. Amyloid is also seen in association with an extramedullary plasmacytoma.⁷⁸

BENIGN NEOPLASMS**Pleomorphic Adenoma**

Pleomorphic adenoma (PA) is the most common tumor in all salivary glands, in children and in adults. Two thirds of parotid tumors and 50% of all salivary gland tumors

are PAs. The most commonly encountered site is the superficial parotid, often the tail of the gland at the angle of the jaw. On physical examination, the nodule is firm, reflecting the abundance of chondromyxoid matrix. The aspiration is typically painless.

CYTOMORPHOLOGY OF PLEOMORPHIC ADENOMA:

- epithelial cells
- myoepithelial cells
- chondromyxoid matrix
- tyrosine crystalloids (non-specific)

The epithelial cells are recognizable by their cohesive groupings, usually in a honeycomb pattern. When present as individual cells, epithelial cells are indistinguishable from myoepithelial cells. The myoepithelial cells have a variety of appearances: spindle shaped (the most common), epithelioid, clear-cell, and plasmacytoid. The plasmacytoid morphology is particularly common in palatal tumors. Unlike epithelial cells, myoepithelial cells are commonly found individually, within matrix material, in loose clusters, or in larger, haphazardly arranged clusters. The matrix material is the most characteristic finding.^{79,80} Fibrillary and “chondromyxoid” in appearance, it stains pale purple/blue in Papanicolaou-stained preparations and can be difficult to see and to distinguish from other extracellular material such as mucin (Fig. 10.8A). It is best appreciated on air-dried preparations stained with a Romanowsky-type stain, which gives it an intense magenta color that is “metachromatic,”

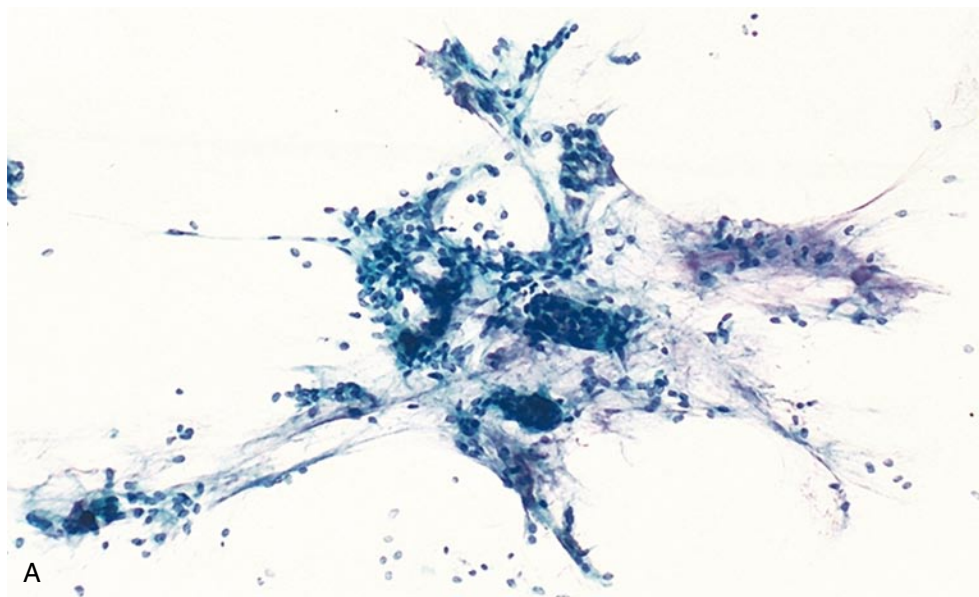


Figure 10.8 Pleomorphic adenoma. A, Pale-blue matrix, myoepithelial cells, and sheets of ductal cells are identified at low power (Papanicolaou stain).

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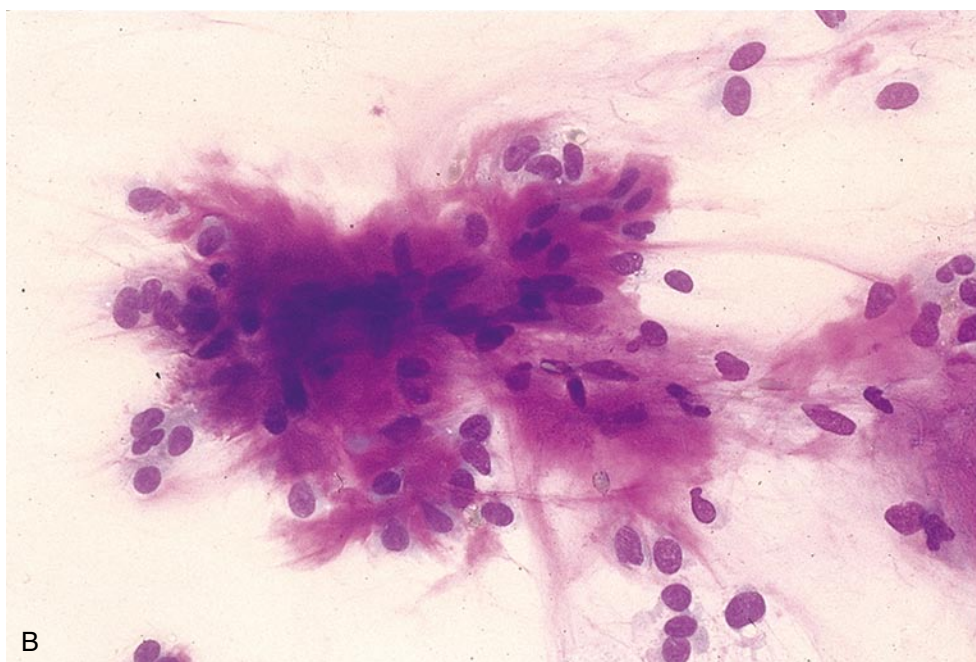


Figure 10.8 (Continued) **Pleomorphic adenoma.** *B*, In the air-dried preparation, magenta-colored fibrillary matrix has frayed edges. Isolated plump spindle-shaped myoepithelial cells are embedded within the matrix (Romanowsky stain).

meaning that the dye in the stain changes color through its interaction with the matrix material (Fig. 10.8*B*). Although the matrix of adenoid cystic carcinoma stains with a similar intensity, the features are otherwise distinctive.^{79,81} The distinctive fibrillary nature of the matrix in a PA can be appreciated by its frayed, indistinct margins. Myoepithelial cells are readily identified embedded within it.

The proportions of the cellular constituents are extremely variable from one PA to another, and within an individual tumor, hence the importance of sampling different areas of the tumor with multiple passes. In at least two thirds of cases, the typical constituents are readily appreciated, without unusual features, and the diagnosis is straightforward.⁸²

Pitfalls Associated with Pleomorphic Adenomas

Sparse or absent matrix. The absence or paucity of matrix may preclude a definitive diagnosis of PA,^{80,83} but an appropriate differential diagnosis can result in proper clinical management. The differential diagnosis includes basal cell adenoma or adenocarcinoma in epithelial-rich lesions and myoepithelioma in myoepithelial-rich lesions.

Adenoid cystic-like matrix. Cylindromatous areas, raising the possibility of adenoid cystic carcinoma, are encountered in approximately 5% of PAs⁸² (Fig. 10.9*A* and *B*). If a well-sampled lesion is otherwise typical for a

PA, and these cylindromatous areas are rare, this finding is of no concern. If extensive, the diagnosis of adenoid cystic carcinoma, or the rare adenoid cystic carcinoma ex pleomorphic adenoma,⁸⁴ should be considered.

Cytologic atypia. Focal, mild cytologic atypia is seen in 20% of PAs⁸² and causes little concern. Severe atypia, however, particularly if accompanied by necrosis or abundant mitoses, raises the possibility of carcinoma ex pleomorphic adenoma. Most carcinomas ex pleomorphic adenoma are cytologically overtly malignant, and the challenge is recognizing the preexisting PA. Extensive atypia without overt malignant features (Fig. 10.10) justifies the diagnosis of a PA with atypia, with an accompanying comment that the finding could be indicative of early malignant change.

Squamous or mucinous metaplasia. These changes are of little concern when present focally in an otherwise typical PA. If extensive, the differential diagnosis includes mucoepidermoid carcinoma, possibly arising in a preexisting PA.^{85,86} Mucoepidermoid carcinoma ex pleomorphic adenoma is exceedingly rare and usually a high-grade malignancy.⁸⁷

Myoepithelioma

Myoepithelioma is a rare and unusual salivary gland tumor that can be considered a monomorphic variant of pleomorphic adenoma. The clinical features and natural history of myoepitheliomas and pleomorphic adenomas are similar.³⁸

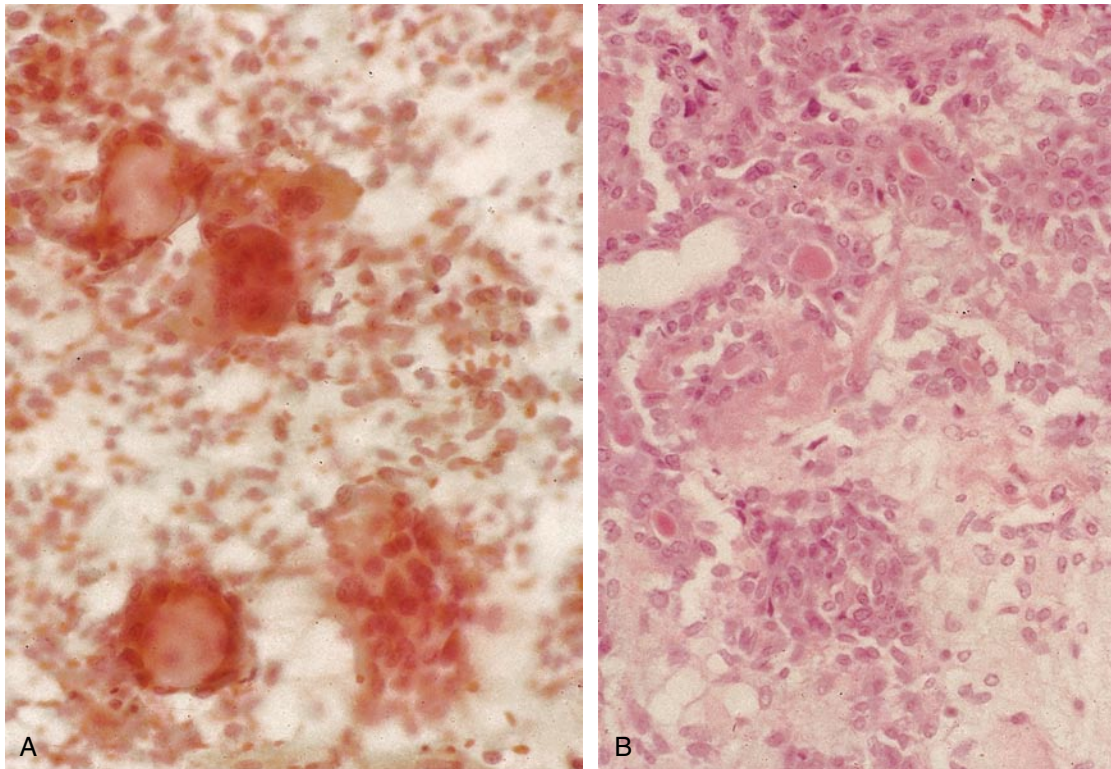


Figure 10.9 Pleomorphic adenoma with adenoid cystic-like foci. *A*, Lucent cylinders are present in a background of myoepithelial cells (Papanicolaou stain). *B*, In the corresponding histologic specimen, cylinders are also seen. The findings in the aspirate and resected specimen were otherwise typical of a pleomorphic adenoma (hematoxylin-eosin [H & E] stain).

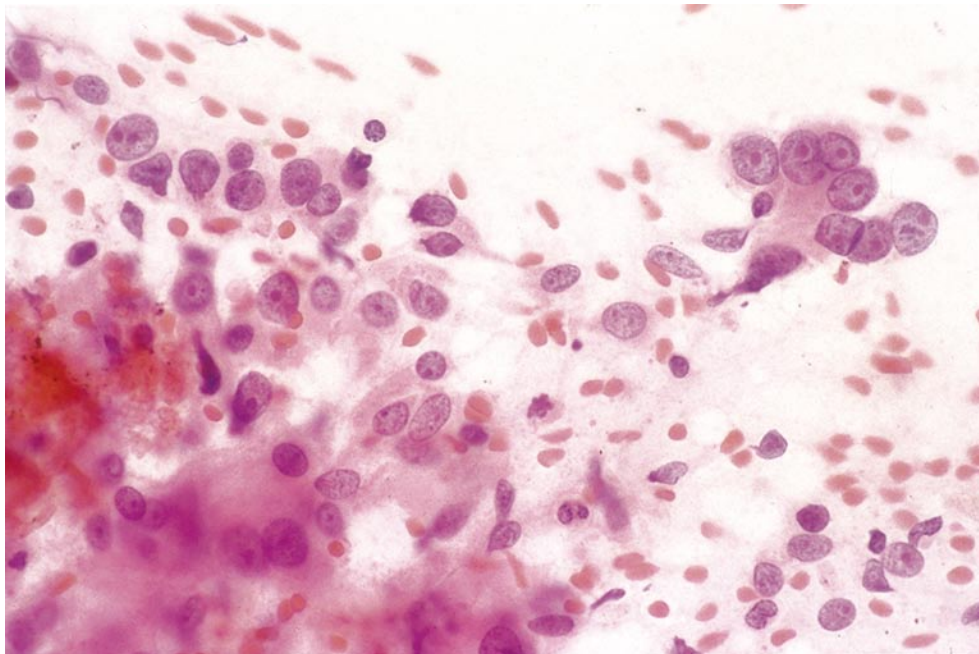


Figure 10.10 Pleomorphic adenoma with atypia. Atypical cells with enlarged nuclei and prominent nucleoli are present in an aspirate that is otherwise typical of a pleomorphic adenoma. When few and far between, such cells are not concerning for malignancy. More extensive atypia, as in this case, merits the interpretation pleomorphic adenoma with atypia. The resected specimen revealed a noninvasive carcinoma ex pleomorphic adenoma (hematoxylin-eosin [H & E] stain).

CYTOMORPHOLOGY OF MYOEPITHELIOMA:

- loose aggregates and isolated cells
- spindle-cell, clear-cell, epithelioid, or plasmacytoid morphology
- lack of honeycomb-like sheets of epithelial cells
- lack of chondromyxoid matrix

The myoepithelial cells of a myoepithelioma are identical to those of a pleomorphic adenoma. The absence of chondromyxoid matrix material and epithelial cells distinguishes a myoepithelioma from a pleomorphic adenoma. A smear composed exclusively of myoepithelial cells, however, often results from incomplete sampling of a myoepithelial-rich pleomorphic adenoma⁸³ (Fig. 10.11) and can result in a misdiagnosis of myoepithelioma. Although this error has no clinical consequence, a generic interpretation of a “myoepithelial cell-rich neoplasm” (accompanied by a differential diagnosis) is prudent in such circumstances.

A spindle cell myoepithelioma can be difficult to distinguish from a schwannoma.^{83,88-90} Both can have nuclear palisading, although this is more common in a schwannoma, in which rows of palisaded nuclei are called *Verrocay bodies*. Immunohistochemistry is helpful, although S-100 is diffusely positive in schwannoma and often positive in myoepithelial cells. Myoepithelial cells also stain for one or more of the following: p63, keratins, smooth muscle actin, glial fibrillary acidic protein, and calponin. Of these, p63 has the greatest use of the current immunohistochemical markers of myoepithelial differentiation. An important caveat is that p63 also

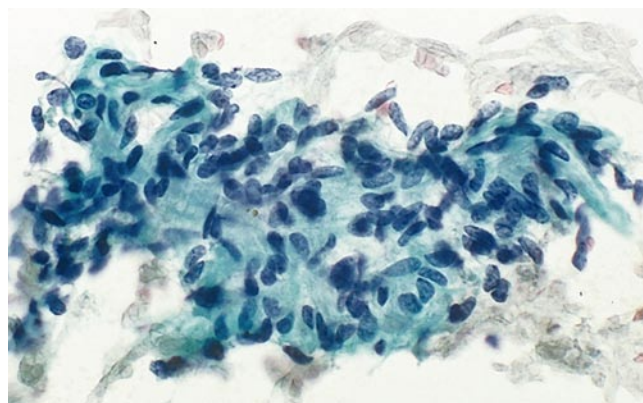


Figure 10.11 Myoepithelial cell-rich neoplasm. Loosely cohesive, spindle-shaped myoepithelial cells are the sole constituents of this aspirate. The resected specimen proved to be a myoepithelial-rich pleomorphic adenoma, but the aspiration findings were indistinguishable from those of a myoepithelioma (Papanicolaou stain).

stains cells with squamous differentiation (e.g., the epidermoid cells of a mucoepidermoid carcinoma).

Plasmacytoid myoepithelial tumors^{91,92} (Fig. 10.12) resemble plasmacytomas, but myoepithelial cells lack the “clock-face” chromatin and perinuclear hof of plasma cells. Immunohistochemistry and serum electrophoresis are useful in difficult cases.

Clear cell myoepithelioma resembles other neoplasms with clear-cell differentiation, like epithelial-myoepithelial carcinoma, acinic cell carcinoma, mucoepidermoid carcinoma, and metastatic renal cell carcinoma.

Myoepithelial carcinoma is the rare malignant counterpart. Necrosis, pleomorphism, mitotic activity, coarse chromatin, and prominent nucleoli are distinguishing features.⁹³

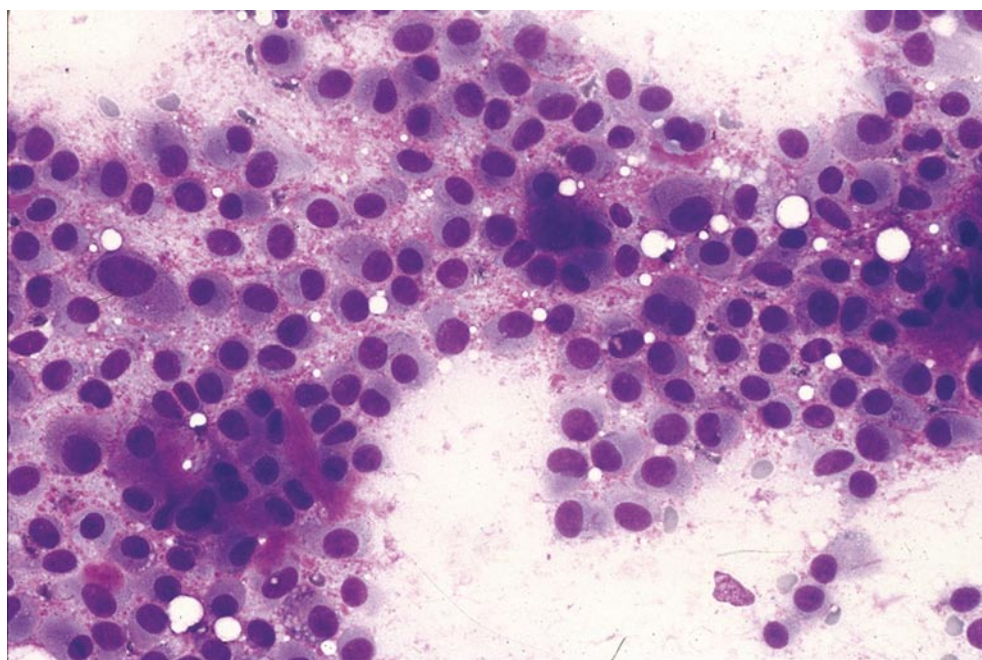


Figure 10.12 Plasmacytoid myoepithelial cells in a pleomorphic adenoma. Round to oval myoepithelial cells with eccentric nuclei mimic plasma cells, but wisps of matrix material are present, and the perinuclear hof and “clock-face” chromatin of plasma cells are absent (Romanowsky stain).

Basal Cell Adenoma

Basal cell adenoma occurs most frequently in the parotid gland and clinically is usually mistaken for a pleomorphic adenoma. It can exhibit a variety of histologic patterns: solid, tubular, trabecular, membranous, and mixed patterns. The canalicular adenoma is a distinct entity because of its propensity for the upper lip. The membranous type of basal cell adenoma also has clinically distinct features; unlike the other subtypes, it is associated with an autosomal dominant syndrome that includes the development of other synchronous cutaneous tumors.⁹⁴

CYTOMORPHOLOGY OF BASAL CELL ADENOMA:

- small-sized and intermediate-sized basaloid cells with peripheral palisading
- dense, nonfibrillary stroma at the periphery of cell groups

Sheets and cords of neoplastic cells are arranged in large, geographic configurations with a sharply demarcated epithelial-stromal interface. The matrix of a basal

cell adenoma stains brightly cyanophilic or eosinophilic with the Papanicolaou stain, and basophilic to metachromatic with Romanowsky-type stains. The neoplastic cells, of epithelial and myoepithelial derivation, have a “basaloid” appearance (i.e., scant cytoplasm and uniform round to oval nuclei). Bare nuclei can be abundant. Two populations of basaloid cells, arranged in variable-sized clusters, are often present, sometimes with peripheral palisading of the smaller cells^{95,96} (Fig. 10.13A). Many cell groups show a peripheral band of hyaline material and sparse intercellular hyaline matrix that is not fibrillar (as in pleomorphic adenoma). Smooth-contoured hyaline globules may be present.⁹⁷⁻⁹⁹ The globules or cylinders, small and often interdigitated with basaloid myoepithelial cells, are a nonspecific finding associated with any myoepithelial-containing tumor (Fig. 10.13B). The small globules or cylinders of a basal cell adenoma differ from the variably sized, often large, individual spheres commonly seen in adenoid cystic carcinoma.

The *membranous type* (also called the *dermal analogue tumor*) is a cytologically and clinically distinct variant in which coalescing spheres of matrix material are prominent, and aggregates of basaloid cells are surrounded by a thick ribbon of matrix material^{97,100-102}

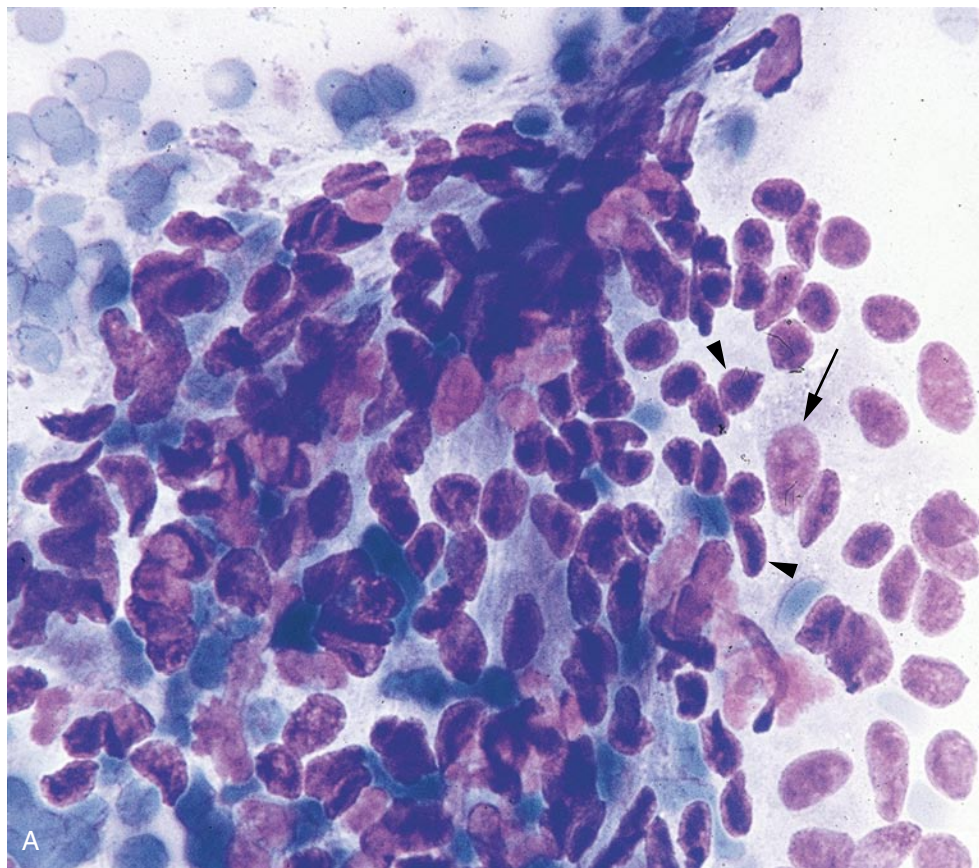


Figure 10.13 Basaloid neoplasms. A, Two populations of loosely cohesive cells are present: cells with larger nuclei and moderate amounts of cytoplasm (arrow) and a second population of basaloid cells with dark nuclei and scant cytoplasm (arrowheads). A more specific interpretation than basaloid neoplasm is not possible. Histologic examination revealed a basal cell adenocarcinoma (Romanowsky stain).

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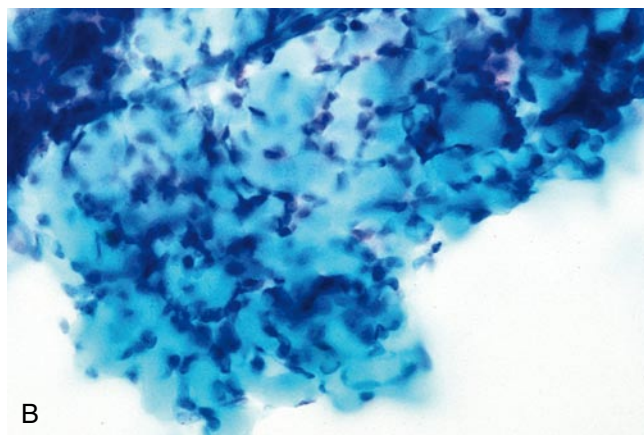


Figure 10.13 (Continued) **Basaloid neoplasms.** B, A lattice-like network of myoepithelial cells surrounds coalescing extracellular matrix globules. This pattern is non-specific and seen in a variety of matrix-containing basaloid neoplasms. Histologic examination revealed a pleomorphic adenoma (Papanicolaou stain).

(Fig. 10.14A and B). It is indistinguishable from a dermal cylindroma and resembles a basaloid squamous cell carcinoma. In most instances, however, a basaloid squamous cell carcinoma exhibits cytologic features of malignancy, including necrosis, marked atypia, and mitotic activity. HPV is associated with a subset of basaloid squamous cell carcinomas, and in difficult cases a positive HPV test helps make the distinction.

DIFFERENTIAL DIAGNOSIS OF BASALOID NEOPLASMS:

- chronic sialadenitis
- basal cell adenoma
- basal cell adenocarcinoma
- adenoid cystic carcinoma (solid variant)

- cellular pleomorphic adenoma
- metastatic basal cell carcinoma
- metastatic basaloid squamous cell carcinoma
- small cell carcinoma

Evaluating and interpreting the basaloid neoplasms is the most difficult problem in salivary gland FNA. The differential diagnosis includes chronic sialadenitis, benign tumors, low-grade malignancies, and high-grade malignancies. The solid variant of adenoid cystic carcinoma, basal cell carcinoma of the skin,¹⁰³ metastatic and primary small cell carcinomas, and rare malignant basaloid tumors—basaloid squamous cell carcinoma,¹⁰⁴⁻¹⁰⁶ and basal cell adenocarcinoma^{96,107-110}—are all in the differential diagnosis.

Unlike the other conditions on this list, chronic sialadenitis is typically sparsely cellular and has a background of chronic inflammation. It is usually a diffuse process rather than a discrete mass. Although cytologic atypia, necrosis, and significant mitotic activity exclude basal cell adenoma, the absence of these malignant features does not exclude a malignancy like adenoid cystic carcinoma^{98,99} and basal cell adenocarcinoma. By FNA, basal cell adenoma cannot be distinguished from its low-grade malignant counterpart (basal cell adenocarcinoma), as the diagnosis rests on the histologic demonstration of an infiltrative growth pattern. Both basal cell adenoma and the solid variant of adenoid cystic carcinoma can contain occasional stromal cylinders like those of the usual type of adenoid cystic carcinoma. Stromal material in basal cell adenomas often surrounds the neoplastic cells, in contrast to adenoid cystic carcinoma, in which the neoplastic cells almost always surround stroma. The matrix of basal cell adenoma can be hyalinized and show dense staining characteristics with the Papanicolaou

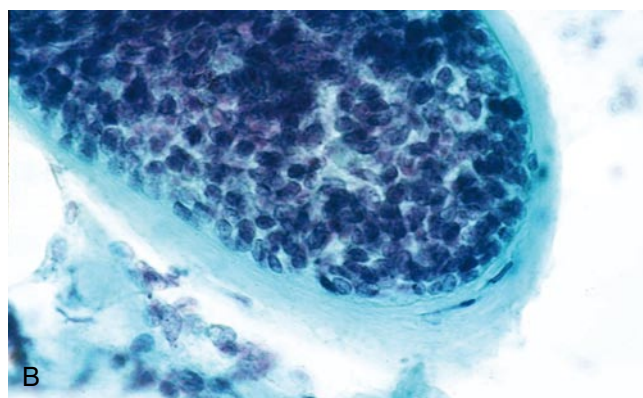


Figure 10.14 **Basal cell adenoma, membranous type.** A, Clusters of basaloid cells are surrounded by a ribbon (“cuticle”) of dense aqua-colored matrix material (arrow; Papanicolaou stain). B, The ribbon of matrix is better seen in this high magnification image (Papanicolaou stain).

stain, whereas in adenoid cystic carcinoma the matrix is more typically transparent. Squamous whorls are a histologic feature of basal cell adenoma, but they are an uncommon finding in FNA samples. Nevertheless, when present, squamous whorls help to exclude an adenoid cystic carcinoma, which never exhibits squamous differentiation. Clinical evidence of malignancy and a complete history can also be helpful in distinguishing these entities.

In many cases a specific diagnosis is not possible, but a differential diagnosis can help guide clinical management (see sample reports herein). Frozen section diagnosis at the time of excision can also assist in guiding the extent of surgery.

Reporting basaloid neoplasms—sample reports:

1. If the tumor has characteristic features (e.g., peripheral band of hyaline material) of basal cell adenoma/adenocarcinoma:
NEOPLASTIC CELLS PRESENT.
Basal cell neoplasm of salivary gland origin (see Note).

NOTE: The cytologic features are compatible with either basal cell adenoma or basal cell adenocarcinoma. Distinction between the two cannot be made on cytologic material. Excision is recommended for precise classification.

2. If the tumor lacks distinctive cytologic features:
NEOPLASTIC CELLS PRESENT.
Basaloid neoplasm (see Note).

NOTE: The differential diagnosis is broad and includes basal cell adenoma, basal cell adenocarcinoma, the solid variant of adenoid cystic carcinoma, and nonsalivary gland neoplasms. Excision is recommended for precise classification, including intraoperative frozen section examination if clinically indicated.

Warthin Tumor

Warthin tumor (WT) occurs primarily in the parotid gland and periparotid region and is believed to arise from salivary duct remnants entrapped within salivary gland-associated lymph nodes.³⁸ It is the second most common salivary gland neoplasm, representing approximately 5% to 10% of all salivary gland tumors. It occurs most often in the 50- to 79-year age range, is more common in men, and can be bilateral.³⁸ WTs feel “doughy” on palpation and contain distinctive thick, brown-green, granular fluid grossly resembling motor oil.

CYTOMORPHOLOGY OF WARTHIN TUMOR:

- lymphocytes
- oncocytes
- granular debris

The typical aspirate is composed of lymphocytes, oncocytes, and granular debris⁸⁰ (Fig. 10.15). The lymphocyte population usually predominates, and is comprised mostly of small lymphocytes, with some admixed larger, reactive forms. Mast cells are present and are easiest to appreciate in Romanowsky-stained preparations.¹¹¹ Oncocytes, which may be scant, form cohesive flat sheets with an orderly arrangement of cells, usually without three-dimensional crowding. Infrequently, papillary groups and even a bilayered arrangement, as seen in histologic sections, are found. The abundant cytoplasm of oncocytes looks different depending on what stain is used. The cytoplasm is granular, orange-pink to grey-blue with the Papanicolaou stain, but waxy (non-granular) and deep blue with the Romanowsky-type stains. Nuclei are usually uniform, round and eccentrically located, with evenly textured chromatin and small nucleoli, but some can be enlarged, with prominent nucleoli. Nuclear enlargement and prominence of nucleoli are not signs of malignancy, however, in WTs.

In some cases, especially those associated with marked inflammation, squamous or mucinous metaplasia is present, suggesting a diagnosis of squamous cell carcinoma or mucoepidermoid carcinoma.^{56,112} In such cases, a careful search reveals the characteristic duo of lymphocytes and oncocytes. Other entities in the differential diagnosis include oncocytoma, acinic cell carcinoma, and metastatic renal cell carcinoma. Occasionally, aspirates of WTs are unsatisfactory or

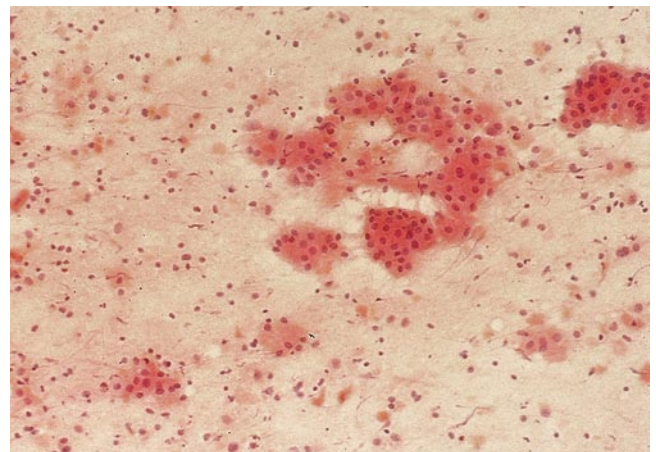


Figure 10.15 Warthin tumor. Scattered, small cohesive clusters of oncocytes are present in a background of lymphocytes (Papanicolaou stain).

result in a nonspecific diagnosis. Because WT is a cystic neoplasm, sampling sometimes yields cyst contents only. In other cases a spontaneous, partial or complete infarction may have occurred, resulting in necrosis and few, if any, intact, viable cells.

Oncocytoma

Oncocytoma is a rare benign salivary gland neoplasm comprising 1% to 3% of all salivary gland tumors.^{38,113} In contrast to oncocytic metaplasia and oncocytosis, oncocytoma is a discrete, clinically detectable nodule. It is well-circumscribed and demarcated from the surrounding salivary gland parenchyma by at least a partial fibrous capsule.³⁸ Most oncocytomas occur in the parotid gland, and the peak incidence is in the seventh to ninth decades; these tumors are rare in patients under 50 years of age.^{38,114}

CYTOMORPHOLOGY OF ONCOCYTOMA:

- cellular specimen
- oncocytes
- clean background
- no lymphocytes

Aspirates are moderately cellular and consist of large polygonal oncocytes whose cytoplasm, like that of the WT oncocytes, contains an abundance of mitochondria¹¹⁴ (Fig. 10.16A and B). Oncocytes are present as sheets, cords, dyshesive clusters and single cells with sharp cellular outlines. As with the oncocytes of a WT, the abundant cytoplasm looks different depending on

what stain is used. The cytoplasm is granular, orange-pink to gray-blue with the Papanicolaou stain, but waxy (non-granular) and deep blue with the Romanowsky-type stains. Nuclei are usually small, round to oval and centrally placed with small nucleoli; variable nuclear enlargement, vesiculation and distinct nucleoli may be seen, but mitotic activity is absent. The background lacks the granular proteinaceous debris and lymphocytes of a WT.

CYTOMORPHOLOGY OF ONCOCYTOMA:

- cellular specimen
- oncocytes
- clean background
- no lymphocytes

DIFFERENTIAL DIAGNOSIS OF ONCOCYTIC LESIONS:

- oncocytoma
- oncocytosis
- Warthin tumor
- pleomorphic adenoma
- mucoepidermoid carcinoma
- acinic cell carcinoma
- oncocytic carcinoma
- metastatic renal cell carcinoma

Because oncocytes and oncocytic change are present in normal salivary glands and a variety of salivary gland neoplasms, the differential diagnosis is broad.¹¹⁵ Oncocytes occur in oncocytic metaplasia, oncocytosis, and oncocytoma,¹¹⁵ and in some cases, it can be

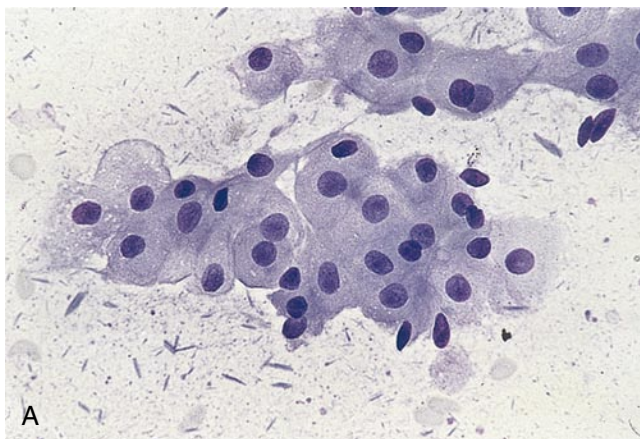


Figure 10.16 Oncocytoma. A, Cohesive sheet-like clusters of oncocytes with distinct cell borders are present in a clean background (Romanowsky stain). B, Oncocytes have uniform round to oval nuclei and abundant densely granular cytoplasm (Papanicolaou stain).

difficult or impossible to distinguish between these entities cytologically, particularly oncocytosis and oncocytoma. WT is distinguished from an oncocytoma by virtue of its abundant lymphocytes and relatively minor component of oncocytes. With regard to the rare oncocytic carcinoma, cytologic features alone do not always allow for distinction from an oncocytoma, although marked cytologic atypia, mitotic activity, necrosis, or appropriate clinical symptoms alert one to the possibility of an oncocytic carcinoma.^{116,117} Oncocytic carcinoma is a diagnosis of exclusion and must be distinguished from other tumors with oncocytic change. The rare oncocytic variant of mucoepidermoid carcinoma is identified by a mixture of mucin-producing and intermediate cell types.

Although the finely granular or waxy cytoplasm of oncocytomas and WT differs from the vacuolated cytoplasm of acinic cell carcinoma, these tumors have overlapping cytologic features, especially at low magnification, where acinic cell carcinomas appear to consist of sheets of cells with dense cytoplasm and uniform nuclei.¹¹⁸ The challenge is exacerbated by the existence of clear cell variants of both oncocytoma and acinic cell carcinoma. Special stains can be helpful: the mitochondria of oncocytes are strongly positive with a phosphotungstic acid hematoxylin (PTAH) stain, whereas acinic cell carcinoma is negative; conversely, oncocytes lack the characteristic PAS-positive, diastase-resistant cytoplasmic granules of an acinic cell carcinoma. We have seen several instances in which the distinction between acinar and oncocytic differentiation was not possible by examining only smear preparations, but characteristic acinar cell cytoplasm was readily apparent in cell block material, without the need for special stains (see Fig. 10.19D). In difficult cases, a diagnosis of a *low-grade salivary gland neoplasm* with the appropriate differential diagnosis is justified.

CARCINOMAS OF SALIVARY GLAND ORIGIN

Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma (MEC) is the most common salivary gland malignancy in children and adults, and the most common malignancy of the major and minor salivary glands. Clinical, histologic, and cytologic features differ between the low- and high-grade tumors. Low-grade MECs are more commonly cystic, whereas high-grade MECs are solid and infiltrative. Modest surgical excision is curative for low-grade MECs; high-grade MECs require aggressive surgical treatment, and sometimes adjunctive radiotherapy and lymph node dissection.

CYTOMORPHOLOGY OF MUCOEPIDERMOID CARCINOMA:

- mucus cells (predominate in low-grade tumors)
- epidermoid cells (predominate in high-grade tumors)
- intermediate cells
- extracellular mucin
- overt cytologic malignancy (high-grade tumors)

The diagnostic feature of MEC is the combination of mucus cells, epidermoid (squamous) cells, and intermediate cells, with clear cells often present as well.¹¹⁹⁻¹²² Mucus cells have a columnar or signet ring-like appearance; the epidermoid cells are polygonal and squamous, with dense cyanophilic cytoplasm; and the intermediate cells are smaller, resembling immature squamous metaplastic cells. In some instances, individual cells can combine features of both mucinous and epidermoid differentiation; such cells are considered diagnostic of MEC. Clear cells in MEC represent a morphologic variant of squamous differentiation.³⁸

Low-grade MEC is commonly cystic, and mucus cells predominate (Fig. 10.17A). Individual cells with features of both mucinous and epidermoid differentiation are seen and are considered diagnostic (Fig. 10.17B). Overt cytologic evidence of malignancy is not seen, and because the low-grade tumors are cystic, the neoplastic cells are sometimes under sampled. As a result, low-grade MEC is the most frequent cause of a false-negative diagnosis in salivary gland FNA.¹²⁰ In some instances, only extracellular mucin—pale purple with Romanowsky stains, pale blue-purple with the Papanicolaou stain—is seen. Scattered, round neoplastic mucinous cells may be mistaken for histiocytes.

DIFFERENTIAL DIAGNOSIS OF LOW-GRADE MUCOEPIDERMOID CARCINOMA:

- acquired cyst (mucocele or retention cyst)
- chronic sialadenitis with mucinous metaplasia of ducts
- Warthin tumor
- pleomorphic adenoma

The differential diagnosis of low-grade MEC includes an acquired cyst (mucocele or retention cyst) and ductal mucinous metaplasia in chronic sialadenitis (commonly associated with sialolithiasis). The presence of intermediate or epidermoid cells excludes an acquired cyst. Stone fragments are diagnostic of sialolithiasis, and ciliated mucinous columnar cells are a metaplastic change seen with duct obstruction. If these features are not apparent, a low-grade MEC cannot be excluded. Thus, whenever a specimen contains extracellular mucin, an atypical

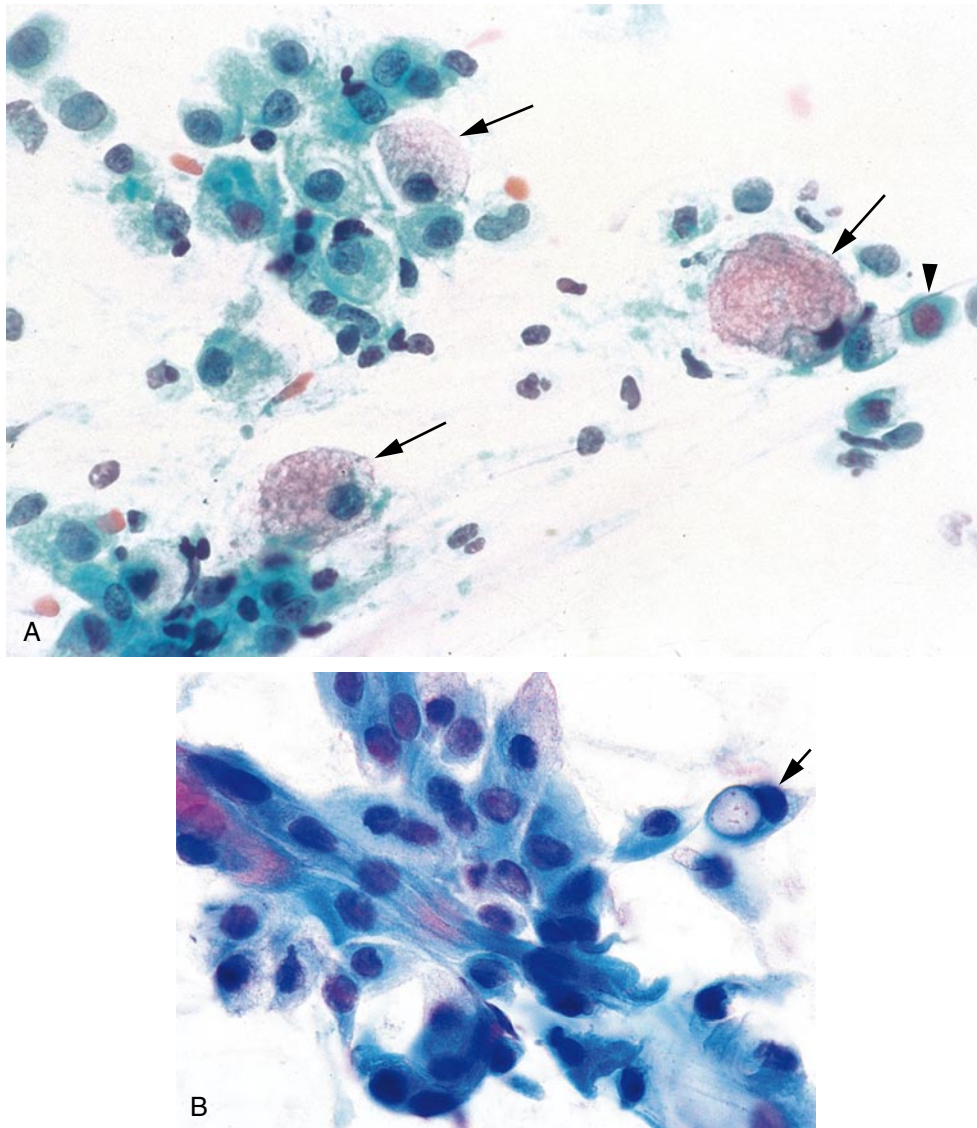


Figure 10.17 Low-grade mucoepidermoid carcinoma (MEC). A, In a low-grade MEC, mucus cells (arrows) and intermediate cells (arrowhead) predominate (Papanicolaou stain). B, In this low-grade MEC, squamoid cells predominate. There is an isolated cell with features of both squamoid and mucinous differentiation (arrow; Papanicolaou stain).

(or suspicious) interpretation is warranted, with an appropriate explanatory note. As with all cystic lesions, any residual mass should be reaspirated after drainage, and excision should be considered if the lesion persists.

Distinction of low-grade MEC from a WT is more problematic because oncocytic variants of MEC do occur. Atypia and nononcocyctic epithelial cells can be seen in both, but are more common in MEC. In an otherwise typical pleomorphic adenoma, small foci of squamous or mucinous differentiation are not significant. MEC ex pleomorphic adenoma is extremely rare and is usually a high-grade malignancy.⁸⁵⁻⁸⁷

High-grade MEC has a greater proportion of squamoid cells, with more cytologic atypia, and a less prominent cystic component. Recognizing the tumor as a

malignancy is straightforward (Fig. 10.18); precise identification as a high-grade MEC can be difficult.

DIFFERENTIAL DIAGNOSIS OF HIGH-GRADE MUCOEPIDERMOID CARCINOMA:

- carcinoma ex pleomorphic adenoma
- salivary duct carcinoma
- oncocytic carcinoma
- metastatic carcinoma

High-grade carcinomas in the salivary gland include high-grade MEC, carcinoma ex pleomorphic adenoma, salivary duct carcinoma, oncocytic carcinoma, and metastatic carcinoma, most frequently metastatic squamous

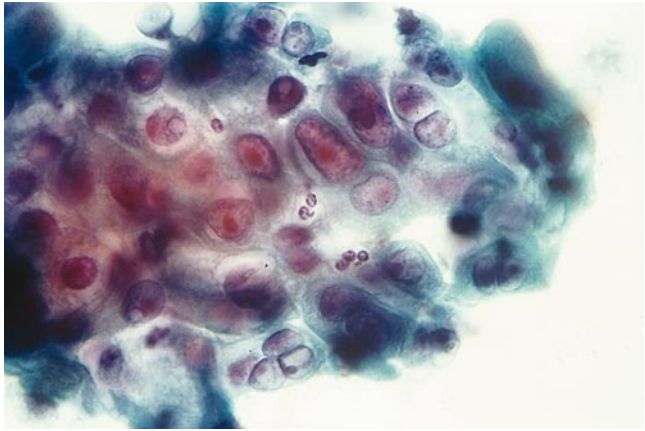


Figure 10.18 High-grade mucoepidermoid carcinoma (MEC). In a high-grade MEC, squamoid cells predominate. Marked nuclear atypia is present (Papanicolaou stain).

cell carcinoma. On rare occasions, any salivary gland carcinoma, in fact, can undergo dedifferentiation and take on the appearance of a high-grade malignancy. Primary high-grade salivary gland carcinomas are treated similarly, so the distinction is not critical for patient management. A high-grade MEC is recognized by the combination of squamoid, mucinous, and intermediate-type cells. Distinction from a nonkeratinizing squamous cell carcinoma is dependent on the identification of a mucinous component. A mucicarmine stain is diagnostically useful. Carcinoma ex pleomorphic adenoma is identified if the benign component (pleomorphic adenoma) is apparent. Salivary duct carcinoma has extensive necrosis and can have a papillary architecture. Oncocytic carcinoma resembles the oncocytic variant of MEC, but it lacks intracellular mucin. Clinical history is essential to exclude metastatic squamous cell carcinoma. Extensive keratinization (see Fig. 10.6B) is not a feature of high-grade MEC^{4,122} and should prompt a search for a primary squamous cell carcinoma elsewhere, especially in the head and neck region. When lacking specific diagnostic features, the interpretation high-grade carcinoma is sufficient to ensure appropriate therapy.

Acinic Cell Carcinoma

Acinic cell carcinoma is the second most common salivary gland malignancy, representing approximately 4% to 6% of all salivary gland neoplasms and up to 17% of malignancies.^{38,123} It is usually a low-grade malignancy that can recur locally or metastasize to regional lymph nodes and distant sites. The majority arise in the parotid gland, most commonly in women. The mean age of patients is 44 years, although the range is broad and includes children and the elderly.^{38,124} In up to 3% of cases, acinic cell carcinomas are bilateral. Acinic cell carcinoma typically presents as a circumscribed, mobile, slowly growing mass, which occasionally is painful.

CYTOMORPHOLOGY OF ACINIC CELL CARCINOMA:

- cellular smear with serous-type acinar cells:
 - sheets, crowded clusters, isolated cells
 - large polygonal cells, abundant vacuolated cytoplasm
 - PAS-positive diastase-resistant cytoplasmic zymogen granules
 - indistinct cell borders
 - bland nuclei
- naked nuclei with or without lymphocytes

FNA yields a cellular aspirate containing large, polygonal, serous cells with delicate, vacuolated, basophilic cytoplasm (Fig. 10.19A-D). In the background are scattered naked nuclei and sometimes lymphocytes (approximately 10% of cases).¹²⁵⁻¹²⁷ The neoplastic cells have indistinct cell borders and are loosely arranged in three-dimensional groups and sheets. In some cases, a papillary or follicular microarchitecture is present. The cytoplasm may contain occasional coarse zymogen granules that are blue on Papanicolaou-stained preparations and metachromatic with Romanowsky stains. Some cells show marked cytoplasmic vacuolization (see Fig. 10.19C). The cytoplasmic granules are PAS positive and diastase resistant, a characteristic feature that can be assessed, particularly if cell block material is available. The nuclei are usually bland, and nucleoli are sometimes present. Intranuclear pseudoinclusions can be seen; mitoses are infrequent. Some cases contain a predominance of intercalated duct cells. Uncommonly, dedifferentiation occurs in acinic cell carcinoma, manifested by more marked nuclear atypia and less acinar cell differentiation.¹²⁸ This subgroup is particularly difficult to distinguish from other high-grade carcinomas that involve the salivary gland. The rare papillary cystic variant is characterized by papillary groups with vacuolated, histiocyte-like cells that may contain pigment, granular cells, and cyst fluid. These subtle tumors may result in a false-negative interpretation or a mistaken diagnosis of MEC.^{129,130} Acinic cell carcinomas are richly vascular neoplasms, and aspirates often contain delicate blood vessels surrounded by neoplastic cells (see Fig. 10.19A); care should be taken not to mistake these vascular structures for normal ducts. Uncommon features include marked clear cell change, psammoma bodies, and cystic change.

DIFFERENTIAL DIAGNOSIS OF ACINIC CELL CARCINOMA

- normal salivary gland
- sialadenosis
- oncocytoma

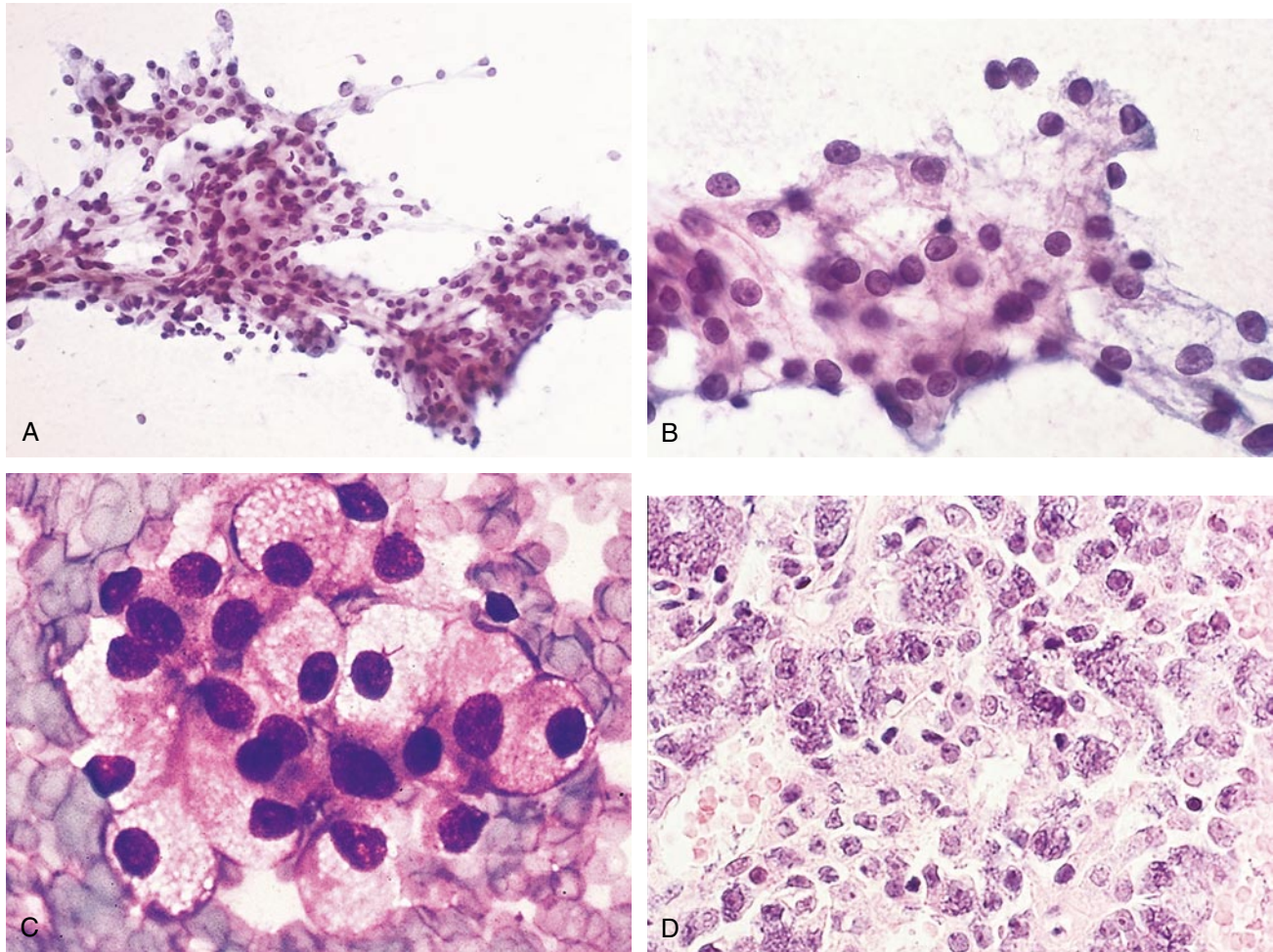


Figure 10.19 Acinic cell carcinoma. A, Loosely cohesive, crowded clusters of cells with acinar differentiation surround thin-walled blood vessels. Note the absence of tightly formed grape-like clusters, adipose tissue, and ductal elements typical of a normal aspirate (compare with Fig. 10.2B; Papanicolaou stain). B, Neoplastic cells have abundant delicate cytoplasm and cytologically bland nuclei (Papanicolaou stain). C, A well-differentiated acinic cell carcinoma displaying abundant vacuolated cytoplasm and small round to oval nuclei. Ductal cells are absent (Romanowsky stain). D, In some instances, acinic cell differentiation is more apparent in cell block preparations, which reveal the typical purple, granular cytoplasm of these cells (hematoxylin-eosin [H & E] stain).

- Warthin tumor
- mucoepidermoid carcinoma
- sebaceous neoplasms
- clear cell neoplasms

Acinic cell carcinoma is distinguished from normal salivary gland and sialadenosis by the looser clustering, overlapping, and dyshesion of the neoplastic acinar cells. Neoplastic cells lack the fibroadipose tissue and cohesive grape-like arrangement of peripherally polarized normal acinar cells surrounding ductal structures typical of normal salivary gland tissue. The distinction from oncocytic neoplasms like WT has been previously discussed. Although the cells of acinic cell carcinoma can be vacuolated or clear and mimic the mucin-containing cells of MEC, the cells of acinic cell carcinoma are negative for mucin. Similarly, the vacuolated cells of acinic cell carcinoma resemble the sebaceous cells of sebaceous lymph-

adenoma and sebaceous carcinoma, but sebaceous cells contain lipid and are PAS positive and diastase sensitive. Acinic cell carcinoma can have a prominent clear cell appearance and thus can mimic other clear cell neoplasms.

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma trails MEC and acinic cell carcinoma in frequency in the major salivary glands, although it occurs at a higher frequency in the submandibular gland. In minor salivary glands, the frequency of adenoid cystic carcinoma is less than previously thought, with the recognition of polymorphous low-grade adenocarcinoma as a distinct pathologic entity.³⁸ Although the clinical course of adenoid cystic carcinoma is often protracted, long-term (10- to 20-year) survival is poor. Three variants are recognized and often present in combination: tubular, cribriform, and solid.

Recognition of the solid pattern is important because of its more aggressive clinical course. The tendency of these tumors to invade nerves manifests itself clinically as a painful mass or as pain during the FNA, which should increase suspicion for malignancy.

CYTOMORPHOLOGY OF ADENOID CYSTIC CARCINOMA:

- variably sized, often large, three-dimensional, acellular hyaline matrix globules and linear branching structures
- matrix is acellular with sharp borders
- basaloid cells
- numerous basaloid cells ($\pm$ atypia) and scant matrix (solid variant)

The cells are basaloid, with scant cytoplasm and hyperchromatic, often angulated, nuclei.¹³¹⁻¹³³ Nucleoli, mitoses, and necrosis are not prominent except in rare

high-grade examples. Most tumors have an abundant acellular matrix that is arranged in discrete globules and cylinders with sharp borders (Figs. 10.20A and B, 10.21). The globules are intensely metachromatic with a Romanowsky stain but nearly invisible with the Papanicolaou stain. The solid variant of adenoid cystic carcinoma has scant matrix, and basaloid cells predominate (Fig. 10.22). Consequently, distinguishing the aggressive solid variant of adenoid cystic carcinoma from basal cell adenoma is often impossible.^{97-99,133,134}

DIFFERENTIAL DIAGNOSIS OF ADENOID CYSTIC CARCINOMA:

- pleomorphic adenoma
- other basaloid neoplasms
- polymorphous low-grade adenocarcinoma
- epithelial-myoepithelial carcinoma
- eccrine cylindroma of the skin

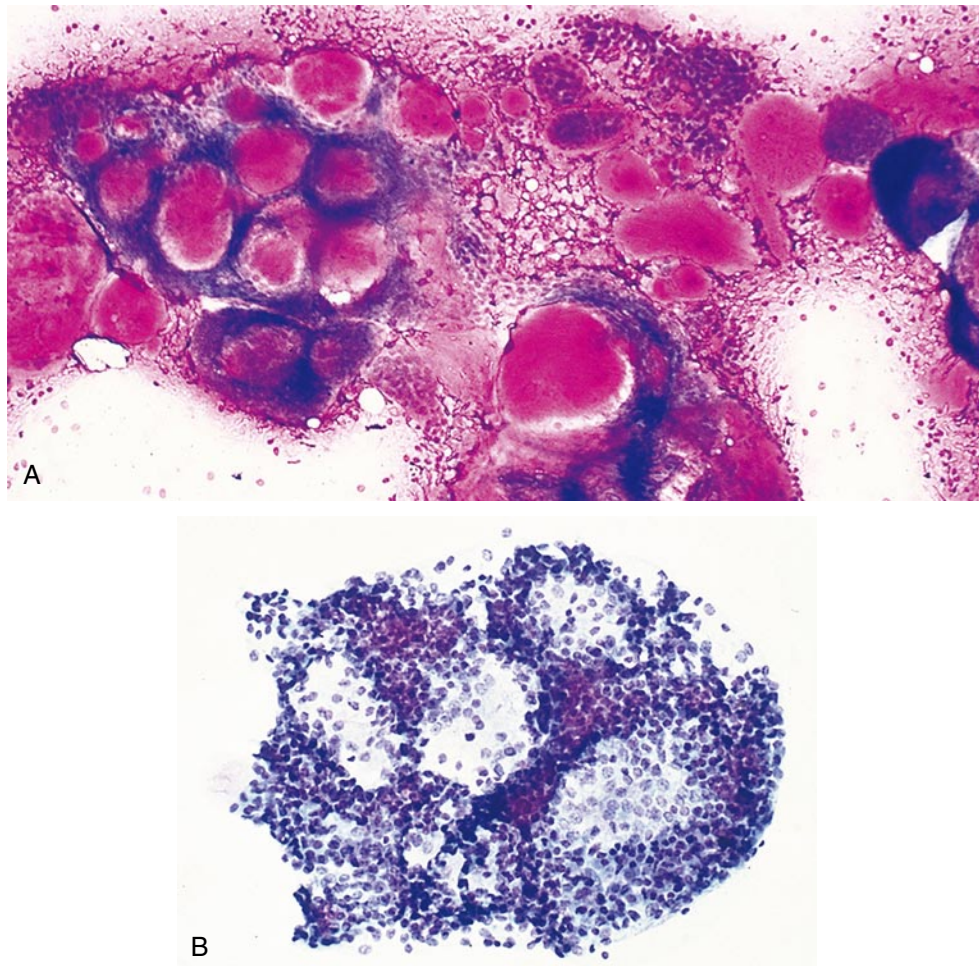


Figure 10.20 Adenoid cystic carcinoma. A, Numerous variably sized stromal spheres are evident (Romanowsky stain). B, With the Papanicolaou stain, the same tumor shows cribriform architecture. The basaloid tumor cells are readily apparent, but the matrix material is transparent (Papanicolaou stain).

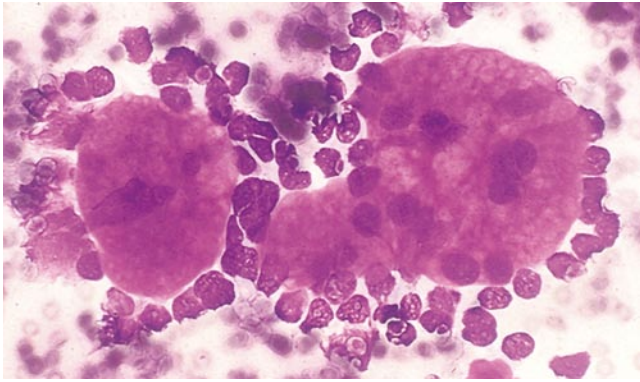


Figure 10.21 Adenoid cystic carcinoma. Basaloid cells surround a matrix that has sharp borders with a "cookie-cutter"-like appearance (Romanowsky stain). (Courtesy of Dr. Imad Nasser, Beth Israel Deaconess Medical Center, Boston)

Although most adenoid cystic carcinomas are straight forward to diagnose by FNA, occasional diagnostic challenges arise. The characteristic acellular spheres of adenoid cystic carcinoma are seen in a variety of other salivary gland tumors, including pleomorphic adenoma, basal cell adenoma, polymorphous low-grade adenocarcinoma, and epithelial-myoepithelial carcinoma,¹³³ but usually they are less numerous or smaller in size in these other neoplasms.

Some PAs contain scattered stromal spheres that resemble those of an adenoid cystic carcinoma. There are other commonalities between the two. For example, the matrix of an adenoid cystic carcinoma is intensely metachromatic with a Romanowsky stain, as is the more usual, chondromyxoid matrix of a PA. On Papanicolaou-

stained preparations, the matrix of both tumors can be nearly invisible. In contrast to the fibrillary quality and embedded myoepithelial cells of the chondromyxoid matrix of a PA, however, the matrix of adenoid cystic carcinoma has sharp edges, as if it were cut out with a cookie cutter. Moreover, the neoplastic cells of adenoid cystic carcinoma surround the matrix material but are not embedded in it. This results in discrete islands of variable-sized globules and fingerlike projections of matrix material in adenoid cystic carcinoma. Less frequently, the matrix of adenoid cystic carcinoma stains densely in Papanicolaou preparations, similar to that of other basaloid tumors like basal cell adenoma and myoepithelioma. In basal cell adenoma and myoepithelioma, however, the matrix islands and surrounding cells form incompletely separated, interdigitating strands.

Polymorphous low-grade adenocarcinoma can have stromal spheres like adenoid cystic carcinoma, but the nuclei are not hyperchromatic. In addition, adenoid cystic carcinoma tends to contain a greater proportion of myoepithelial cells. The key to identifying an epithelial-myoepithelial carcinoma is recognizing a biphasic cell population, an abundance of large clear myoepithelial cells, and the peripherally located acellular basement membrane material. The distinction of adenoid cystic carcinoma from benign dermal eccrine cylindroma can only be made by tumor location (either clinically or radiologically) and not on cytological features.¹³⁵

Given the numerous mimics of adenoid cystic carcinoma and the significant clinical consequences of a diagnosis of adenoid cystic carcinoma, many experts recommend that this diagnosis not be made without clinical evidence of malignancy.^{99,118}

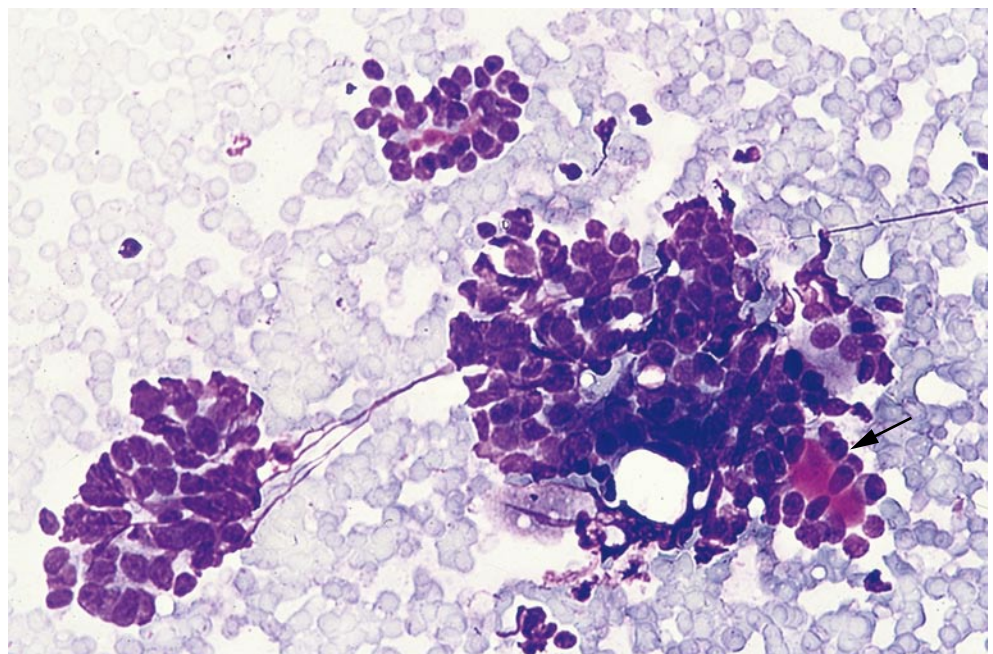


Figure 10.22 Adenoid cystic carcinoma, solid variant. Basaloid cells predominate in this aggressive variant. The identification of rare stromal spheres (arrow) is needed to make the diagnosis (Romanowsky stain).

Malignant Mixed Tumor

In the salivary glands, there are three types of malignant mixed tumor: carcinoma arising in a mixed tumor (carcinoma ex pleomorphic adenoma), metastasizing mixed tumor, and carcinosarcoma.³⁸

Carcinoma arising in a preexisting mixed tumor is the most common form and occurs in approximately 5% to 10% of all mixed tumors. Typically, a longstanding painless mass has undergone rapid enlargement with associated pain (suggestive of perineural involvement by malignancy). In most instances, carcinoma ex pleomorphic adenoma is a high-grade adenocarcinoma of ductal subtype. These tumors are readily recognized as malignant, but they are difficult to distinguish from other high-grade carcinomas such as high-grade MEC, salivary duct carcinoma, and metastatic carcinoma. A preexisting pleomorphic adenoma is necessary for the diagnosis^{136,137} (Fig. 10.23). Conversely, recognition of a preponderance of benign elements should lead to caution in rendering a definitive diagnosis of malignancy.¹³⁶ The typical clinical history may suggest this diagnosis.

A metastasizing mixed tumor is a cytologically benign pleomorphic adenoma in a distant site. Invariably, the primary pleomorphic adenoma of the salivary gland had undergone surgical manipulation, often with multiple local recurrences.¹³⁸ The cytologic features are indistinguishable from pleomorphic adenoma.¹³⁹ Despite its benign appearance, it is often fatal.

True carcinosarcoma occurs rarely and, as is the case at other sites, represents a metaplastic carcinoma. Cytologically, pleomorphic malignant epithelial and

mesenchymal cells are identified.¹⁴⁰⁻¹⁴² The most common differentiated mesenchymal components are chondrosarcoma and osteosarcoma.

Salivary Duct Carcinoma

Salivary duct carcinoma is an uncommon, clinically aggressive malignancy most common in the parotid gland in elderly men. It resembles a high-grade, comedo-type ductal carcinoma of the breast,³⁸ although a rare low-grade variant has been described.^{143,144}

CYTOMORPHOLOGY OF SALIVARY DUCT CARCINOMA:

- overtly malignant cytology
- polygonal cells, abundant granular or vacuolated cytoplasm, prominent nucleoli
- sheets, clusters, papillae, and cribriform groupings
- necrosis

Papillary and cribriform groupings of large, cytologically malignant cells with abundant necrosis strongly suggest the diagnosis (Fig. 10.24A and B).¹⁴⁵⁻¹⁵¹ Distinction from other high-grade malignancies is frequently impossible. This distinction is not of critical importance because aggressive surgical management is recommended for all high-grade primary salivary gland neoplasms. Distinction from a high-grade metastasis to the salivary gland from a primary elsewhere is, however, clinically important. The morphologic distinction may not be possible, and correct interpretation may depend on appropriate clinical information.

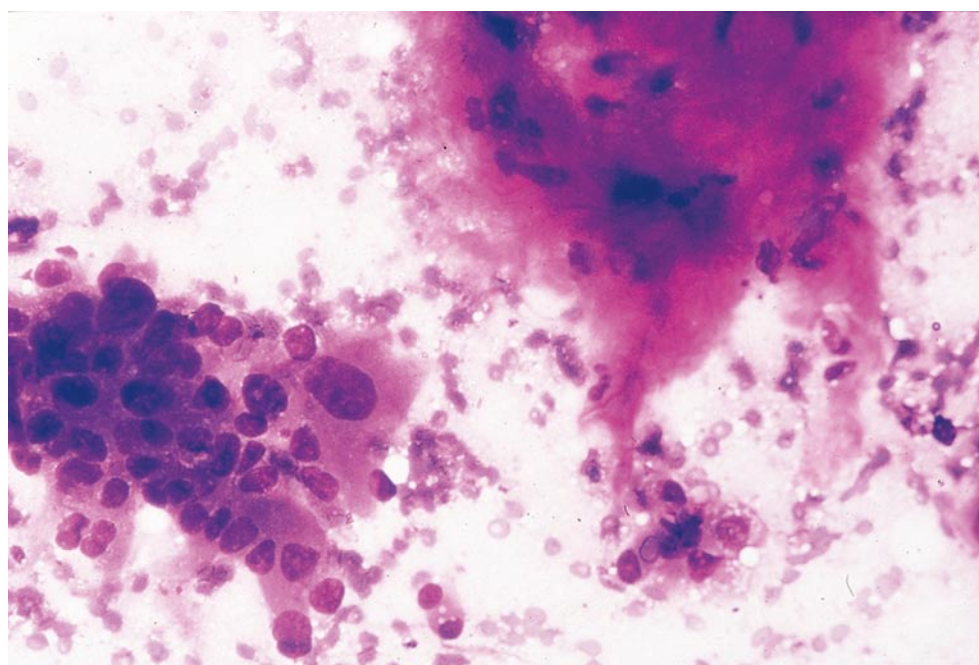


Figure 10.23 Carcinoma ex pleomorphic adenoma. Carcinoma, typically high-grade (lower left), juxtaposed with residual pleomorphic adenoma (upper right) is needed for definitive diagnosis, but the benign elements (well seen here) are often not apparent (Papanicolaou stain).

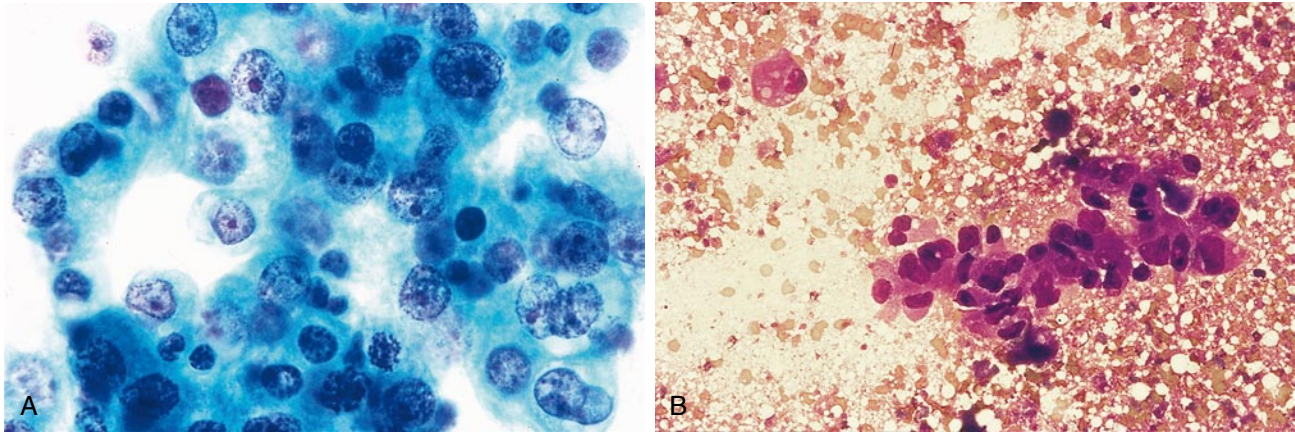


Figure 10.24 High-grade carcinoma (salivary duct carcinoma). A, A high-grade carcinoma is evident on the basis of large, round nuclei with prominent nucleoli and nondescript cytoplasm (Papanicolaou stain). B, A salivary duct carcinoma can be suspected when there is abundant necrosis (Romanowsky stain).

Polymorphous Low-Grade Adenocarcinoma

Polymorphous low-grade adenocarcinoma (PLGA) is a low-grade malignancy characterized by a predilection for perineural invasion, but with an overall favorable prognosis.^{152,153} PLGA is the second most common salivary gland tumor in the minor salivary glands, where this tumor is encountered almost exclusively. Consequently, FNA specimens are rarely encountered. The histologic hallmark is cytologic uniformity with architectural diversity. Cytologically, tubules, cords, and linear cell groupings (like lobular breast carcinoma) are seen.¹⁵⁴⁻¹⁵⁶ The tumor can have stromal spheres like adenoid cystic carcinoma, but the nuclei are normochromatic, not hyperchromatic as in adenoid cystic carcinoma. In addition, adenoid cystic carcinoma tends to contain a greater proportion of myoepithelial cells. A pleomorphic adenoma can be misdiagnosed as a PLGA if fibrillary chondromyxoid stroma is absent. Basal cell adenoma is also in the differential diagnosis, but the clinical features of these entities are distinctive.

RARE MALIGNANT NEOPLASMS

Basal Cell Adenocarcinoma

Basal cell adenocarcinoma is the rare malignant counterpart of basal cell adenoma,^{107,157,158} accounting for 2% of malignant epithelial salivary gland tumors. Most occur in the parotid gland, although occasional cases are encountered in the submandibular and minor salivary glands. Basal cell adenocarcinoma is a low-grade malignancy with a good prognosis but a tendency for local recurrence; metastatic disease is uncommon.

In most instances, basal cell adenocarcinoma is cytologically identical to basal cell adenoma⁹⁶; it is distin-

guished from the latter only histologically, by identifying infiltrative growth. Accordingly, the reader is referred to the previous discussion of basal cell adenoma for the cytologic features and differential diagnosis. A subset shows nuclear atypia, mitotic activity, or necrosis, and is more easily recognized as malignant.³⁸ In these cases, the differential diagnosis is that of basaloid neoplasms with overt malignant features: adenoid cystic carcinoma, metastatic basal cell carcinoma of the skin, metastatic basaloid squamous cell carcinoma, and less commonly, sebaceous carcinoma, Merkel cell carcinoma, and small cell undifferentiated carcinoma.

Epithelial-Myoepithelial Carcinoma

Epithelial-myoepithelial carcinoma is a locally aggressive, low-grade tumor of duct-like structures composed of two cell types: small, inner duct lining cells and larger, peripheral myoepithelial cells. It represents 1% of all salivary gland neoplasms, and over 75% occur in the parotid gland.^{12,38} Although epithelial-myoepithelial carcinomas have a broad age range, the average age at presentation is 62 years. It is twice as common in women as men.^{12,159}

CYTOMORPHOLOGY OF EPITHELIAL-MYOEPITHELIAL CARCINOMA:

- cellular aspirate
- biphasic cell population:
 - large, clear myoepithelial cells
 - small, dark ductal cells
- basement membrane material
- naked nuclei

Three-dimensional clusters of cells are surrounded by small amounts of homogeneous acellular basement membrane material^{160,161} (Fig. 10.25). In some cases, cell clusters surround concentrically laminated

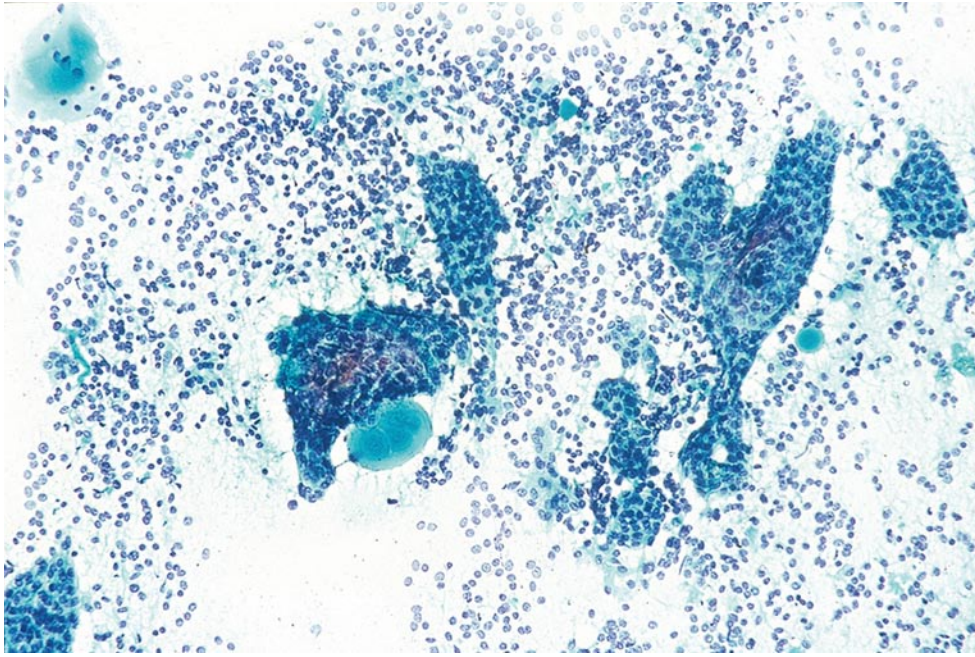


Figure 10.25 Epithelial-myoepithelial carcinoma. Irregular cohesive clusters of epithelial cells are present in a background of bare myoepithelial cell nuclei and acellular hyalinized stromal material (Papanicolaou stain).

proteinaceous spheres, somewhat resembling the matrix spheres of adenoid cystic carcinoma. The epithelial population includes small, dark ductal cells with scant cytoplasm and round to oval nuclei. These are accompanied by a second, often predominant population of large, clear myoepithelial cells with moderate or abundant glycogen-rich cytoplasm, round vesicular nuclei, and small, distinct nucleoli. The background may contain abundant isolated clear cells and naked nuclei, potentially masking the biphasic pattern of the tumor.¹⁶²

DIFFERENTIAL DIAGNOSIS OF EPITHELIAL-MYOEPITHELIAL CARCINOMA:

- adenoid cystic carcinoma
- basaloid neoplasms
- myoepithelial carcinoma
- pleomorphic adenoma
- polymorphous low-grade adenocarcinoma
- metastatic renal cell carcinoma
- other clear cell neoplasms

The key to making the diagnosis of an epithelial-myoepithelial carcinoma is recognizing a biphasic cell population, an abundance of large clear myoepithelial cells, and the peripherally located acellular basement membrane material. The architectural features seen in cell block preparations can be helpful in distinguishing epithelial-myoepithelial carcinoma from other biphasic, epithelial and myoepithelial-derived neoplasms of the salivary gland with overlapping cytologic features.

Clear Cell Carcinoma, Not Otherwise Specified

A number of salivary gland neoplasms can, on rare occasion, exhibit prominent clear cell change.¹⁶³ The rare clear cell carcinoma, not otherwise specified (NOS), a diagnosis of exclusion, is probably the purest example.^{164,165} Optically clear cytoplasm can result from mitochondrial condensation or an accumulation of glycogen, mucin, or fat. The differential diagnosis of a clear cell neoplasm in a salivary gland aspirate is broad.^{163,166,167}

DIFFERENTIAL DIAGNOSIS OF CLEAR CELL CARCINOMA:

- pleomorphic adenoma
- myoepithelioma or myoepithelial carcinoma
- oncocytoma
- lipoma
- acinic cell carcinoma
- epithelial-myoepithelial carcinoma
- mucoepidermoid carcinoma
- metastatic renal cell carcinoma
- sebaceous adenoma or carcinoma

Distinction between these entities is based on an assessment of nuclear atypia, the detection of a second cell population admixed with the clear cells, and a careful search for cells without clear cell features.¹⁶³ Cell block preparations for histochemical stains, to assess the nature of the clear cytoplasm (e.g., glycogen, mucin, zymogen granules), can be especially helpful. Immunohistochemical studies for squamous and myoepithelial differentiation are also helpful.

Primary Small Cell Carcinoma

Primary small cell carcinoma is a rare malignant tumor composed of small, undifferentiated cells, although neuroendocrine features are often present.^{168,169} Because of the morphologic similarity to the more common small cell carcinoma of the lung and cutaneous Merkel cell carcinoma, primary small cell carcinoma of the salivary gland is a diagnosis of exclusion. It represents 1% to 2% of all major salivary gland malignancies. The mean age at presentation is 56 years, and it is much more common in men.¹⁷⁰⁻¹⁷² These are high-grade neoplasms with a poor long-term prognosis.¹⁷⁰

CYTOMORPHOLOGY OF PRIMARY SMALL CELL CARCINOMA:

- cellular smear
- dyshesive, small cells
- round to oval, hyperchromatic nuclei
- indistinct nucleoli
- high nuclear-to-cytoplasmic ratio
- nuclear molding
- frequent mitoses
- necrosis

Aspirates are cytomorphologically indistinguishable from those of a small cell carcinoma of pulmonary origin. Isolated cells and loosely cohesive clusters of cells slightly larger than lymphocytes have a high nuclear-to-cytoplasmic ratio and a hyperchromatic nucleus. Nuclei are round to oval, with dispersed chromatin and indistinct nucleoli. Nuclear molding, mitotic activity, and necrotic material are characteristic.^{173,174}

Special studies are helpful. Immunocytochemical studies show that most cells are positive for cytokeratins, usually with a dot-like pattern. In addition, many tumors demonstrate reactivity for the neuroendocrine markers synaptophysin, chromogranin, Leu-7, and neuron-specific enolase.¹⁷⁰

The differential diagnosis includes lymphoma, the solid variant of adenoid cystic carcinoma, basaloid squamous cell carcinoma, metastatic small cell carcinoma from the respiratory tract, and Merkel cell carcinoma. Most primary small cell carcinomas of the salivary gland (and Merkel cell carcinomas) show positive immunoreactivity for cytokeratin 20, which distinguishes them from small cell carcinomas of pulmonary origin.^{174,175}

Lymphoepithelial Carcinoma

Lymphoepithelial carcinomas are clinically aggressive undifferentiated tumors that are most common among

people from Greenland and southern China or those that are of North American Inuit descent. This tumor comprises less than 0.5% of salivary gland neoplasms³⁸ and is cytologically and histologically similar to its nasopharyngeal counterpart.³⁹ The median age of patients is 40 years.

CYTOMORPHOLOGY OF LYMPHOEPITHELIAL CARCINOMA:

- syncytial sheets of undifferentiated cells
- pleomorphic, vesicular nuclei
- large nucleoli
- numerous mitoses
- lymphocytes and plasma cells

Aspirates contain a mixture of lymphocytes, plasma cells, and syncytial sheets of large, oval to spindle-shaped cells, with malignant cytologic features: markedly pleomorphic, vesicular nuclei with prominent nucleoli, small to moderate amounts of cytoplasm, and abundant mitotic activity.^{17,176-178} The differential diagnosis includes other undifferentiated carcinomas. In particular, metastatic nasopharyngeal carcinoma must be excluded; this is based on clinical rather than cytologic features. Both neoplasms are strongly associated with the Epstein-Barr virus (EBV). Keratin immunostains are helpful to exclude lymphoma and malignant melanoma.

Adenocarcinoma, Not Otherwise Specified

Salivary gland adenocarcinomas without specific features are categorized as adenocarcinoma, NOS. With the increased recognition of specific entities, this category is diminishing.¹⁷⁹ Low-grade adenocarcinomas may not be recognized as malignant because the diagnosis rests on the histologic identification of infiltrative growth. High-grade adenocarcinoma, NOS is recognizably malignant and defined by the absence of the distinctive features of other, specified salivary gland malignancies.

OTHER MALIGNANCIES

Squamous Cell Carcinoma

Squamous cell carcinoma is a rare primary malignancy in the salivary gland. Metastasis to the salivary gland or intraparenchymal lymph nodes is far more common and must be excluded on clinical grounds.

Metastases from head and neck squamous cell carcinomas are often cystic and, therefore, a potentially significant source of false-negative diagnoses. The differential diagnosis was previously discussed. High-grade nonkeratinizing squamous cell carcinoma may be

indistinguishable from other high-grade salivary gland carcinomas, especially MEC. Identification of mucin production is helpful for diagnosing MEC. A history of malignancy elsewhere favors metastasis.

Lymphoma Involving the Salivary Gland

Primary and secondary malignant lymphomas occur in the salivary glands and intraparotid lymph nodes, and constitute 2% to 5% of salivary gland neoplasms.^{66,180} The parotid region is the most frequently involved. Most lymphomas are B-cell non-Hodgkin lymphomas; the most common are extranodal marginal zone lymphoma of the MALT type, follicular lymphoma, and diffuse large B-cell lymphoma.^{39,66} Follicular lymphomas mainly involve periparotid lymph nodes rather than the salivary gland itself. Hodgkin lymphoma rarely involves the salivary glands. A more detailed discussion of the cytologic criteria for diagnosing lymphomas is found in Chapter 11.

CYTOMORPHOLOGY OF MALIGNANT LYMPHOMAS:

- extranodal marginal zone lymphoma of MALT type
 - small- to intermediate-size lymphocytes with pale cytoplasm (monocytoid B-cells)
 - round to slightly irregular nuclei
 - occasional immunoblasts
 - CD45+, CD20+, CD23–, CD10–, CD5–, bcl2+, bcl6–, cyclin D1–
- diffuse large B-cell lymphoma
 - large markedly atypical lymphocytes
 - CD45+, CD20+, keratin–, S-100–

Extranodal marginal zone lymphoma of MALT type is a low-grade B-cell lymphoma that typically develops in a

background of LESA or Sjögren syndrome.^{181,182} It constitutes the majority of primary salivary gland lymphomas. Aspirates contain a mixed population of small-sized to intermediate-sized lymphocytes (monocytoid B cells) with a moderate amount of pale staining cytoplasm, round to slightly irregular nuclei, condensed chromatin and indistinct nucleoli, admixed with occasional immunoblasts^{183,184} (Fig. 10.26A). Tingible-body macrophages are generally scant to absent, but their presence should not be used to exclude the diagnosis of lymphoma. In some cases, the aspirate may exhibit a polymorphous population of lymphocytes, making the cytologic diagnosis of lymphoma quite difficult; immunophenotyping is recommended when considering the diagnosis of any lymphoma, particularly MALT type. The cells of MALT lymphoma are typically CD20+, CD5–, CD10–, CD23–, bcl2+, bcl6–, and cyclin D1 negative.^{66,184} The primary differential diagnosis includes reactive intraparotid lymph nodes, LESA, cystic lymphoid hyperplasia, and chronic sialadenitis. Some epithelial lesions have a prominent lymphoid component (WT, sebaceous lymphadenoma, MEC, acinic cell carcinoma, and lymphoepithelial carcinoma), and may raise the possibility of lymphoma. MALT lymphomas are distinguished from follicular lymphomas by their round to only slightly irregular nuclei and more monomorphic population of cells. In addition, follicular lymphomas are bcl2+, bcl6+, and have a characteristic translocation.

Diffuse large B-cell lymphoma is composed of an overtly malignant population of large centroblastic and immunoblastic cells (Fig. 10.26B). Small cell and undifferentiated malignancies, such as lymphoepithelial carcinoma, malignant melanoma, and small cell carcinoma, must be excluded. Demonstration of lymphoid markers like leukocyte common antigen (CD45) and the B-cell markers CD19 and CD20, along with the absence of cytokeratins and other nonlymphoid markers, is especially helpful in difficult cases.

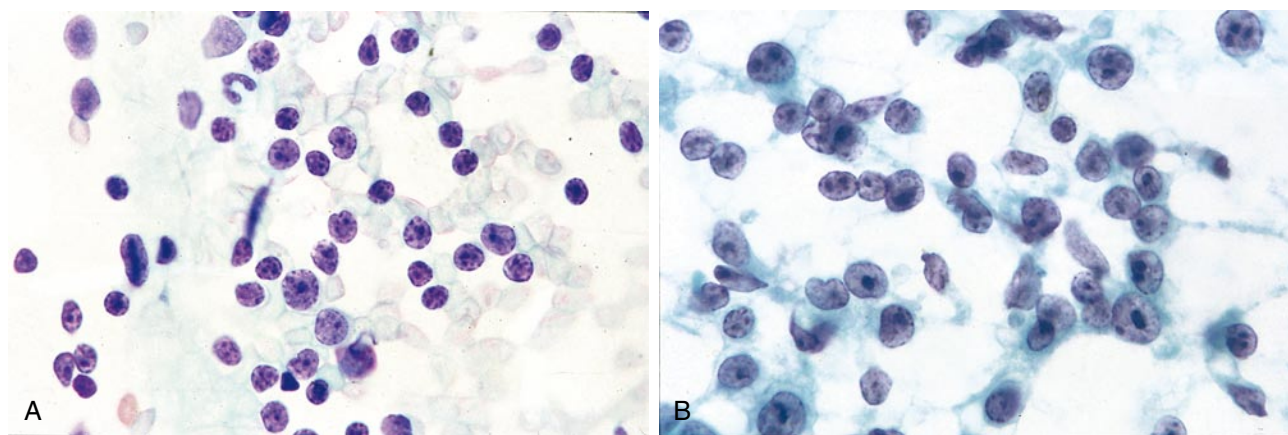


Figure 10.26 Lymphoma. A, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) type. A monomorphic population of small-sized to intermediate-sized lymphocytes with a moderate amount of pale cytoplasm (monocytoid B cells) is characteristic, but confirmatory marker studies are necessary (Papanicolaou stain). B, Diffuse large B-cell lymphoma. There is a predominant population of large, markedly atypical lymphoid cells with distinct nucleoli and nuclear pleomorphism (Papanicolaou stain).

MISCELLANEOUS

Metastatic tumors to the salivary gland or intraparenchymal lymph nodes are not uncommon and are readily documented by FNA.^{185,186} In most instances, the primary site is in the head and neck; metastatic squamous cell carcinoma and malignant melanoma account for the vast majority.

In children, pleomorphic adenoma is the most common neoplasm; MEC and acinic cell carcinoma are the most common malignancies. *Hemangiomas* are the most common salivary gland neoplasm in infants. Aspirates yield blood and bland spindle cells.^{187,188}

Summary for salivary gland

- Salivary gland FNA is an effective tool that helps in the classification, clinical management, and surgical planning of salivary gland lesions.
- Precise classification is possible for many of the commonly encountered lesions, especially pleomorphic adenoma and Warthin tumor.
- Even in the absence of a specific diagnosis, an appropriate differential diagnosis provides clinically useful information.
- The basaloid neoplasms pose the biggest dilemma in clinical management.
- Cystic lesions may result in a false-negative diagnosis, but appropriate clinical communication and follow-up ultimately results in the correct diagnosis for these lesions.
- In all lesions, cystic and solid, a well-sampled, cellular specimen is vital. With sparsely cellular specimens, a conservative approach, with a differential diagnosis rather than an unequivocal interpretation, is strongly encouraged.

REFERENCES

1. Eneroth CM, Frazen S, Zajicek J: Cytologic diagnosis of aspirate from 1000 salivary-gland tumours. *Acta Otolaryngol* 1966;Suppl 224:168-172.
2. Frable MA, Frable WJ: Fine-needle aspiration biopsy of salivary glands. *Laryngoscope* 1991;101:245-249.
3. Heller KS, Dubner S, Chess Q, Attie JN: Value of fine needle aspiration biopsy of salivary gland masses in clinical decision-making. *Am J Surg* 1992;164:667-670.
4. Layfield LJ, Glasgow BJ: Diagnosis of salivary gland tumors by fine-needle aspiration cytology: A review of clinical utility and pitfalls. *Diagn Cytopathol* 1991;7:267-272.
5. Lindberg LG, Akerman M: Aspiration cytology of salivary gland tumors: Diagnostic experience from six years of routine laboratory work. *Laryngoscope* 1976;86:584-594.
6. Persson PS, Zettergren L: Cytologic diagnosis of salivary gland tumors by aspiration biopsy. *Acta Cytol* 1973;17:351-354.
7. Qizilbash AH, Sianos J, Young JE, Archibald SD: Fine needle aspiration biopsy cytology of major salivary glands. *Acta Cytol* 1985;29:503-512.
8. Stewart CJ, MacKenzie K, McGarry GW, Mowat A: Fine-needle aspiration cytology of salivary gland: A review of 341 cases. *Diagn Cytopathol* 2000;22:139-146.
9. Rajwanshi A, Gupta K, Gupta N, et al.: Fine-needle aspiration cytology of salivary glands: Diagnostic pitfalls—revisited. *Diagn Cytopathol* 2006;34:580-584.
10. Layfield LJ, Gopez E, Hirschowitz S: Cost efficiency analysis for fine-needle aspiration in the workup of parotid and submandibular gland nodules. *Diagn Cytopathol* 2006;34(11):734-738.
11. McKean ME, Lee K, McGregor IA: The distribution of lymph nodes in and around the parotid gland: An anatomical study. *Br J Plast Surg* 1985;38:1-5.
12. Batsakis JG, Sneige N, el-Naggar AK: Fine-needle aspiration of salivary glands: Its utility and tissue effects [see comments]. *Ann Otol Rhinol Laryngol* 1992;101:185-188.
13. Spiro RH: Diagnosis and pitfalls in the treatment of parotid tumors. *Semin Surg Oncol* 1991;7:20-24.
14. Martin HE, Ellis EB: Biopsy by needle puncture and aspiration. *Ann Surg* 1930;92:169-181.
15. Martin SE, Ellis EB: Aspiration biopsy. *Surg Gynecol Obstet* 2001;59:578-589.
16. Stewart FW: The diagnosis of tumors by aspiration. *Am J Pathol* 1933;9:801-812.
17. Chan MK, McGuire LJ, King W, et al.: Cytodiagnosis of 112 salivary gland lesions. Correlation with histologic and frozen section diagnosis. *Acta Cytol* 1992;36:353-363.
18. Jandu M, Webster K: The role of operator experience in fine needle aspiration cytology of head and neck masses. *Int J Oral Maxillofac Surg* 1999;28:441-444.
19. Jayaram N, Ashim D, Rajwanshi A, et al.: The value of fine-needle aspiration biopsy in the cytodiagnosis of salivary gland lesions. *Diagn Cytopathol* 1989;5:349-354.
20. Kocjan G, Nayagam M, Harris M: Fine needle aspiration cytology of salivary gland lesions: Advantages and pitfalls. *Cytopathology* 1990;1:269-275.
21. Lau T, Balle VH, Bretlau P: Fine needle aspiration biopsy in salivary gland tumours. *Clin Otolaryngol* 1986;11:75-77.
22. Layfield LJ, Tan P, Glasgow BJ: Fine-needle aspiration of salivary gland lesions. Comparison with frozen sections and histologic findings. *Arch Pathol Lab Med* 1987;111:346-353.
23. Nettle WJ, Orell SR: Fine needle aspiration in the diagnosis of salivary gland lesions. *Aust NZ J Surg* 1989;59:47-51.
24. O'Dwyer P, Farrar WB, James AG, et al.: Needle aspiration biopsy of major salivary gland tumors. Its value. *Cancer* 1986;57:554-557.
25. Roland NJ, Caslin AW, Smith PA, et al.: Fine needle aspiration cytology of salivary gland lesions reported immediately in a head and neck clinic. *J Laryngol Otol* 1993;107:1025-1028.
26. Weinberger MS, Rosenberg WW, Meurer WT, Robbins KT: Fine-needle aspiration of parotid gland lesions. *Head Neck* 1992;14:483-487.
27. Young JA, Smallman LA, Thompson H, et al.: Fine needle aspiration cytology of salivary gland lesions. *Cytopathology* 1990;1:25-33.
28. Geisinger KR, Weidner N: Aspiration cytology of salivary glands. *Semin Diagn Pathol* 1986;3:219-226.
29. Tabbara SO, Frierson HFJ, Fechner RE: Diagnostic problems in tissues previously sampled by fine-needle aspiration. *Am J Clin Pathol* 1991;96:76-80.
30. Allen CM, Damm D, Neville B, et al.: Necrosis in benign salivary gland neoplasms. Not necessarily a sign of malignant transformation. *Oral Surg Oral Med Oral Pathol* 1994;78:455-461.
31. Kern SB: Necrosis of a Warthin's tumor following fine needle aspiration. *Acta Cytol* 1988;32:207-208.
32. Pabuccuoglu HU, Lebe B, Sarioglu S, Lebe E: Infarction of pleomorphic adenoma: A rare complication of fine-needle

- aspiration obscuring definitive diagnosis. *Diagn Cytopathol* 2001;24:301-303.
33. Mukunyadzi P, Bardales RH, Palmer HE, Stanley MW: Tissue effects of salivary gland fine-needle aspiration. Does this procedure preclude accurate histologic diagnosis? *Am J Clin Pathol* 2000;114:741-745.
 34. Al-Khafaji BM, Afify AM: Salivary gland fine needle aspiration using the ThinPrep technique: Diagnostic accuracy, cytologic artifacts and pitfalls. *Acta Cytol* 2001;45:567-574.
 35. Michael CW, Hunter B: Interpretation of fine-needle aspirates processed by the ThinPrep technique: Cytologic artifacts and diagnostic pitfalls. *Diagn Cytopathol* 2000;23:6-13.
 36. Parfitt JR, McLachlin CM, Weir MM: Comparison of ThinPrep and conventional smears in salivary gland fine-needle aspiration biopsies. *Cancer* 2007;111(2):123-129.
 37. Stanley MW: Selected problems in fine needle aspiration of head and neck masses. *Mod Pathol* 2002;15(3):342-350.
 38. Ellis GL, Auclair PL: Tumors of the Salivary Glands. Washington DC, American Registry of Pathology 2008.
 39. Barnes L, Eveson JW, Reichart P, Sidransky D (eds): World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. Lyon, France, IARC Press, 2005.
 40. Witt RL: Major salivary gland cancer. *Surg Oncol Clin N Am* 2004;13(1):113-127.
 41. Pantanowitz L, Goulart R, Cao JQ: Salivary gland crystalloids. *Diagn Cytopathol* 2006;34:749-750.
 42. Saenz-Santamaria J, Catalina-Fernandez I, Fernandez-Mera JJ: Sialadenitis with crystalloid formation: Fine needle aspiration cytodiagnosis of 15 cases. *Acta Cytol* 2003;47:1-4.
 43. Carson HJ, Raslan WF, Castelli MJ, Gattuso P: Tyrosine crystals in benign parotid gland cysts: report of two cases diagnosed by fine-needle aspiration biopsy with ultrastructural and histochemical evaluation. *Am J Clin Pathol* 1994;102:699-702.
 44. Cleveland DB, Cosgrove MM, Martin SE: Tyrosine-rich crystalloids in a fine needle aspirate of a polymorphous low grade adenocarcinoma of a minor salivary gland. A case report. *Acta Cytol* 1994;38:247-251.
 45. Gilcrease MZ, Nelson FS, Guzman-Paz M: Tyrosine-rich crystals associated with oncocytic salivary gland neoplasms. *Arch Pathol Lab Med* 1998;122:644-649.
 46. Lemos LB, Baliga M, Brister T, Cason Z: Cytomorphology of tyrosine-rich crystalloids in fine needle aspirates of salivary gland adenomas. *Acta Cytol* 1997;41:1709-1713.
 47. Granter SR, Renshaw AA, Cibas ES: Nontyrosine crystalloids in fine-needle aspiration specimens of the parotid gland: a report of two cases and review of the literature. *Diagn Cytopathol* 1999;20:44-46.
 48. Nasuti JE, Gupta PK, Fleisher SR, LiVolsi VA: Nontyrosine crystalloids in salivary gland lesions: Report of seven cases with fine-needle aspiration cytology and follow-up surgical pathology. *Diagn Cytopathol* 2000;22:167-171.
 49. Boutonnat J, Ducros V, Pinel C, et al.: Identification of amylase crystalloids in cystic lesions of the parotid gland. *Acta Cytol* 2000;44(1):51-56.
 50. Lopez-Rios F, Diaz-Bustamante T, Serrano-Egea A, et al.: Amylase crystalloids in salivary gland lesions: Report of a case with a review of the literature. *Diagn Cytopathol* 2001;25(1):59-62.
 51. Henry-Stanley MJ, Beneke J, Bardales RH, Stanley MW: Fine-needle aspiration of normal tissue from enlarged salivary glands: Sialosis or missed target? *Diagn Cytopathol* 1995;13:300-303.
 52. Droese M: Cytological diagnosis of sialadenitis, sialadenitis, and parotid cysts by fine-needle aspiration biopsy. *Adv Otorhinolaryngol* 1981;26:49-96.
 53. Stanley MW, Bardales RH, Beneke J, et al.: Sialolithiasis. Differential diagnostic problems in fine-needle aspiration cytology. *Am J Clin Pathol* 1996;106:229-233.
 54. Namiq AL, Tollefson T, Fan F: Cryptococcal parotitis presenting as a cystic parotid mass: Report of a case diagnosed by fine-needle aspiration cytology. *Diagn Cytopathol* 2005;33:36-38.
 55. Reyes CV, Jensen JD: Parotid abscess due to salmonella enteritidis: A case report. *Acta Cytol* 2006;50:677-679.
 56. Mooney EE, Dodd LG, Layfield LJ: Squamous cells in fine-needle aspiration biopsies of salivary gland lesions: Potential pitfalls in cytologic diagnosis. *Diagn Cytopathol* 1996;15:447-452.
 57. Cheuk W, Chan JK: Kuttner tumor of the submandibular gland: Fine-needle aspiration cytologic findings of seven cases. *Am J Clin Pathol* 2002;117:103-108.
 58. Ochoa ER, Harris NL, Pilch BZ: Marginal zone B-cell lymphoma of the salivary gland arising in chronic sclerosing sialadenitis (Kuttner tumor). *Am J Surg Pathol* 2001;25:1546-1550.
 59. Kaba S, Kojima M, Matsuda H, et al.: Kuttner's tumor of the submandibular glands: Report of five cases with fine-needle aspiration cytology. *Diagn Cytopathol* 2006;34(9):631-635.
 60. Aggarwal AP, Jayaram G, Mandal AK: Sarcoidosis diagnosed on fine-needle aspiration cytology of salivary glands: A report of three cases. *Diagn Cytopathol* 1989;5:289-292.
 61. Frable MA, Frable WJ: Fine-needle aspiration biopsy: Efficacy in the diagnosis of head and neck sarcoidosis. *Laryngoscope* 1984;94:1281-1283.
 62. Mair S, Leiman G, Levinsohn D: Fine needle aspiration cytology of parotid sarcoidosis. *Acta Cytol* 1989;33:169-172.
 63. Perez-Guillermo M, Sola PJ, Espinosa PF: Asteroid bodies and calcium oxalate crystals: Two infrequent findings in fine-needle aspirates of parotid sarcoidosis. *Diagn Cytopathol* 1992;8:248-252.
 64. Ascoli V, Albedi FM, De Blasiis R, Nardi F: Sialadenitis of the parotid gland: Report of four cases diagnosed by fine-needle aspiration cytology. *Diagn Cytopathol* 1993;9:151-155.
 65. Luna MA: Salivary glands. In Pilch BZ (ed): Head and Neck Surgical Pathology. Philadelphia, Lippincott Williams and Wilkins, 2000, pp. 284-349.
 66. Harris NL: Lymphoid proliferations of the salivary glands. *Am J Clin Pathol* 1999;111:S94-S103.
 67. Schmid U, Helbron D, Lennert K: Primary malignant lymphomas localized in salivary glands. *Histopathology* 1982;6:673-687.
 68. Ferry JA, Harris NL: Lymphoma and lymphoid hyperplasia in head and neck sites. In Pilch BZ (ed): Head and Neck Surgical Pathology. Philadelphia, Lippincott Williams & Wilkins 2000, pp. 476-533.
 69. Wong DS, Wong LY: "Cystic" parotid swelling on FNA: Significance on clinical management. *Otolaryngol Head Neck Surg* 2004;130:593-596.
 70. Layfield LJ: Salivary gland. In Layfield LJ (ed): Cytopathology of the Head and Neck. Chicago, American Society of Clinical Pathologists, 1997, pp. 11-88.
 71. Chai C, Dodd LG, Glasgow BJ, Layfield LJ: Salivary gland lesions with a prominent lymphoid component: Cytologic findings and differential diagnosis by fine-needle aspiration biopsy. *Diagn Cytopathol* 1997;17:183-190.
 72. Elliott JN, Oertel YC: Lymphoepithelial cysts of the salivary glands. Histologic and cytologic features. *Am J Clin Pathol* 1990;93:39-43.
 73. Umudum H, Rezanko T, Dag F, Dogruluk T: Human papillomavirus genome detection by in situ hybridization in fine-needle aspirates of metastatic lesions from head and neck squamous cell carcinomas. *Cancer* 2005;105(3):171-177.
 74. Begum S, Gillison ML, Nicol TL, Westra WH: Detection of human papillomavirus-16 in fine-needle aspirates to determine tumor origin in patients with metastatic squamous cell carcinoma of the head and neck. *Clin Cancer Res* 2007;13(4):1186-1191.

75. Myssiorek D, Alvi A, Bhuiya T: Primary salivary gland amyloidosis causing sicca syndrome. *Ann Otol Rhinol Laryngol* 1992;101:487-490.
76. Nandapalan V, Jones TM, Morar P, et al.: Localized amyloidosis of the parotid gland: A case report and review of the localized amyloidosis of the head and neck. *Head Neck* 1998;20:73-78.
77. Siddiqui MA, Gertz M, Dean D: Amyloid goiter as a manifestation of primary systemic amyloidosis. *Thyroid* 2007;17:77-80.
78. Ustun MO, Ekin N, Payzin B: Extramedullary plasmacytoma of the parotid gland: Report of a case with extensive amyloid deposition masking the cytologic and histopathologic picture. *Acta Cytol* 2001;45:449-453.
79. Kapadia SB, Dusenbery D, Dekker A: Fine needle aspiration of pleomorphic adenoma and adenoid cystic carcinoma of salivary gland origin. *Acta Cytol* 1997;41:487-492.
80. Klijanienko J, Vielh P: Fine-needle sampling of salivary gland lesions. I. Cytology and histology correlation of 412 cases of pleomorphic adenoma. *Diagn Cytopathol* 1996;14:195-200.
81. Lee SS, Cho KJ, Jang JJ, Ham EK: Differential diagnosis of adenoid cystic carcinoma from pleomorphic adenoma of the salivary gland on fine needle aspiration cytology. *Acta Cytol* 1996;40:1246-1252.
82. Viguer JM, Vicandi B, Jimenez-Heffernan JA, et al.: Fine needle aspiration cytology of pleomorphic adenoma. An analysis of 212 cases. *Acta Cytol* 1997;41:786-794.
83. Elsheikh TM, Bernacki EG: Fine needle aspiration cytology of cellular pleomorphic adenoma. *Acta Cytol* 1996;40:1165-1175.
84. Geisinger KR, Reynolds GD, Vance RP, McGuirt WF: Adenoid cystic carcinoma arising in a pleomorphic adenoma of the parotid gland. An aspiration cytology and ultrastructural study. *Acta Cytol* 1985;29:522-526.
85. Jacobs JC: Low grade mucoepidermoid carcinoma ex pleomorphic adenoma. A diagnostic problem in fine needle aspiration biopsy. *Acta Cytol* 1994;38:93-97.
86. Pitman MB: Mucoepidermoid carcinoma ex pleomorphic adenoma of the parotid gland. *Acta Cytol* 1995;39:604-606.
87. Klijanienko J, el-Naggar AK, Servois V, et al.: Mucoepidermoid carcinoma ex pleomorphic adenoma: Nonspecific preoperative cytologic findings in six cases. *Cancer* 1998;84:231-234.
88. Cajulis RS, Gokaslan ST, Yu GH, Frias-Hidvegi D: Fine needle aspiration biopsy of the salivary glands. A five-year experience with emphasis on diagnostic pitfalls. *Acta Cytol* 1997;41:1412-1420.
89. Chhieng DC, Cohen JM, Cangiarella JF: Fine-needle aspiration of spindle cell and mesenchymal lesions of the salivary glands. *Diagn Cytopathol* 2000;23:253-259.
90. Dodd LG, Caraway NP, Luna MA, Byers RM: Myoepithelioma of the parotid. Report of a case initially examined by fine needle aspiration biopsy. *Acta Cytol* 1994;38:417-421.
91. Lopez JJ, Ugalde A, Arostegui J, Bilbao FJ: Plasmacytoid myoepithelioma of the soft palate. Report of a case with cytologic, immunohistochemical and electron microscopic studies. *Acta Cytol* 2000;44:647-652.
92. Schultenover SJ, McDonald EC, Ramzy I: Hyaline-cell pleomorphic adenoma. Diagnosis by fine needle aspiration biopsy. *Acta Cytol* 1984;28:593-597.
93. Darvishian F, Lin O: Myoepithelial cell-rich neoplasms: Cytologic features of benign and malignant lesions. *Cancer* 2004;102(6):355-361.
94. Batsakis JG, Luna MA, el-Naggar AK: Basaloid monomorphic adenomas. *Ann Otol Rhinol Laryngol* 1991;100:687-690.
95. Hruban RH, Erozan YS, Zinreich SJ, Kashima HK: Fine-needle aspiration cytology of monomorphic adenomas. *Am J Clin Pathol* 1988;90:46-51.
96. Moroz K, Ferreira C, Dhurandhar N: Fine needle aspiration of basal cell adenocarcinoma of the parotid gland. Report of a case with assessment of DNA ploidy in aspirates and tissue sections by image analysis. *Acta Cytol* 1996;40:773-778.
97. Hood IC, Qizilbash AH, Salama SS, Alexopoulou I: Basal-cell adenoma of parotid. Difficulty of differentiation from adenoid cystic carcinoma on aspiration biopsy. *Acta Cytol* 1983;27:515-520.
98. Stanley MW, Horwitz CA, Henry MJ, et al.: Basal-cell adenoma of the salivary gland: A benign adenoma that cytologically mimics adenoid cystic carcinoma. *Diagn Cytopathol* 1988;4:342-346.
99. Stanley MW, Horwitz CA, Rollins SD, et al.: Basal cell (monomorphic) and minimally pleomorphic adenomas of the salivary glands. Distinction from the solid (anaplastic) type of adenoid cystic carcinoma in fine-needle aspiration. *Am J Clin Pathol* 1996;106:35-41.
100. Gupta RK: Aspiration cytodiagnosis of dermal analogue tumor, a rare subtype of salivary gland monomorphic adenoma: a case report. *Acta Cytol* 1996;40:331-334.
101. Sparrow SA, Frost FA: Salivary monomorphic adenomas of dermal analogue type: Report of two cases. *Diagn Cytopathol* 1993;9:300-303.
102. Vera-Alvarez J, Marigil-Gómez M, García-Prats MD, et al.: Dermal analogue tumor of the salivary gland diagnosed by fine needle aspiration cytology. A case report. *Acta Cytol* 2004;48(1):78-82.
103. Stanley MW, Horwitz CA, Bardales RH, et al.: Basal cell carcinoma metastatic to the salivary glands: Differential diagnosis in fine-needle aspiration cytology. *Diagn Cytopathol* 1997;16:247-252.
104. Banks ER, Frierson HFJ, Covell JL: Fine needle aspiration cytologic findings in metastatic basaloid squamous cell carcinoma of the head and neck. *Acta Cytol* 1992;36:126-131.
105. Gilcrease MZ, Guzman-Paz M: Fine-needle aspiration of basaloid squamous carcinoma: A case report with review of differential diagnostic considerations. *Diagn Cytopathol* 1998;19:210-215.
106. Hoang JT, Foss RD, Nowacki MR, Kelly KE: Basaloid squamous cell carcinoma and fine-needle aspiration: a potential diagnostic pitfall. *Otolaryngol Head Neck Surg* 1998;119:655-657.
107. Atula T, Kleini PJ, Donath K, et al.: Basal cell adenocarcinoma of the parotid gland: a case report and review of the literature. *J Laryngol Otol* 1993;107:862-864.
108. Pisharodi LR: Basal cell adenocarcinoma of the salivary gland. Diagnosis by fine-needle aspiration cytology. *Am J Clin Pathol* 1995;103:603-608.
109. Tawfik O, Tsue T, Pantazis C, et al.: Salivary gland neoplasms with basaloid cell features: report of two cases diagnosed by fine-needle aspiration cytology. *Diagn Cytopathol* 1999;21:46-50.
110. Tse GM, To EW, Yuen EH, Chen M: Basal cell adenocarcinoma of the salivary gland: Report of a case with morphology on fine needle aspiration cytology. *Acta Cytol* 2001;45:775-778.
111. Bottles K, Lowhagen T, Miller TR: Mast cells in the aspiration cytology differential diagnosis of adenolymphoma. *Acta Cytol* 1985;29:513-515.
112. Parwani AV, Ali SZ: Diagnostic accuracy and pitfalls in fine-needle aspiration interpretation of Warthin tumor. *Cancer* 2003;99:166-171.
113. Brandwein MS, Huvos AG: Oncocytic tumors of major salivary glands. A study of 68 cases with follow-up of 44 patients. *Am J Surg Pathol* 1991;15:514-528.
114. Eneroth CM, Zajicek J: Aspiration biopsy of salivary gland tumors. II. Morphologic studies on smears and histologic sections from oncocytic tumors (45 cases of papillary cystadenoma lymphomatosum and 4 cases of oncocytoma). *Acta Cytol* 1965;9:355-361.

115. Sherman ME, Magro C, Berry Y, Szyfelbein WM: Oncocytic nodule. An unusual case of a submaxillary gland mass in an elderly patient. *Acta Cytol* 1990;34:827-830.
116. Abdul-Karim FW, Weaver MG: Needle aspiration cytology of an oncocytic carcinoma of the parotid gland. *Diagn Cytopathol* 1991;7:420-422.
117. Laforga JB, Aranda FI: Oncocytic carcinoma of parotid gland: fine-needle aspiration and histologic findings. *Diagn Cytopathol* 1994;11:376-379.
118. Cooper TL: Fine needle aspiration of the head and neck, Part A: Cytopathology of the salivary glands, neck, soft tissue, and skin. In Pilch BZ (ed): *Head and Neck Surgical Pathology*. Philadelphia, Lippincott Williams & Wilkins, 2000, pp. 618-662.
119. Cohen MB, Fisher PE, Holly EA, et al.: Fine needle aspiration biopsy diagnosis of mucoepidermoid carcinoma. Statistical analysis. *Acta Cytol* 1990;34:43-49.
120. Kljanienco J, Vielh P: Fine-needle sampling of salivary gland lesions. IV. Review of 50 cases of mucoepidermoid carcinoma with histologic correlation. *Diagn Cytopathol* 1997;17:92-98.
121. Kumar N, Kapila K, Verma K: Fine needle aspiration cytology of mucoepidermoid carcinoma. A diagnostic problem. *Acta Cytol* 1991;35:357-359.
122. Zajicek J, Eneroth CM, Jakobsson P: Aspiration biopsy of salivary gland tumors. VI. Morphologic studies on smears and histologic sections from mucoepidermoid carcinoma. *Acta Cytol* 1976;20:35-41.
123. Spiro RH, Huvois AG, Strong EW: Acinic cell carcinoma of salivary origin. A clinicopathologic study of 67 cases. *Cancer* 1978;41:924-935.
124. Abrams AM, Cornyn J, Scofield HH, Hansen LS: Acinic cell adenocarcinoma of the major salivary glands: A clinicopathologic study of 77 cases. *Cancer* 1965;18:1145-1162.
125. Kljanienco J, Vielh P: Fine-needle sampling of salivary gland lesions. V. Cytology of 22 cases of acinic cell carcinoma with histologic correlation. *Diagn Cytopathol* 1997;17:347-352.
126. Nagel H, Laskawi R, Buter JJ, et al.: Cytologic diagnosis of acinic-cell carcinoma of salivary glands. *Diagn Cytopathol* 1997;16:402-412.
127. Mehta RP, Faquin WC, Deschler DG: Acinic cell carcinoma of the parotid gland with ductal extension. *Arch Otolaryngol Head Neck Surg* 2004;130:792-793.
128. Gonzalez-Peramato P, Jimenez-Heffernan JA, Lopez-Ferrer P, et al.: Fine needle aspiration cytology of dedifferentiated acinic cell carcinoma of the parotid gland: A case report. *Acta Cytol* 2006;50:105-108.
129. Ali SZ: Acinic-cell carcinoma, papillary-cystic variant: A diagnostic dilemma in salivary gland aspiration. *Diagn Cytopathol* 2002;27(4):244-250.
130. Shet T, Ghodke R, Kane S, Chinoy RN: Cytomorphologic patterns in papillary cystic variant of acinic cell carcinoma of the salivary gland. *Acta Cytol* 2006;50(4):388-392.
131. Eneroth CM, Zajicek J: Aspiration biopsy of salivary gland tumors. IV. Morphologic studies on smears and histologic sections from 45 cases of adenoid cystic carcinoma. *Acta Cytol* 1969;13:59-63.
132. Kljanienco J, Vielh P: Fine-needle sampling of salivary gland lesions. III. Cytologic and histologic correlation of 75 cases of adenoid cystic carcinoma: review and experience at the Institut Curie with emphasis on cytologic pitfalls. *Diagn Cytopathol* 1997;17:36-41.
133. Nagel H, Hotze HJ, Laskawi R, et al.: Cytologic diagnosis of adenoid cystic carcinoma of salivary glands. *Diagn Cytopathol* 1999;20:358-366.
134. Yu GH, Caraway NP: Poorly-differentiated adenoid cystic carcinoma: Cytologic appearance in fine-needle aspirates of distant metastases. *Diagn Cytopathol* 1996;15:296-300.
135. Bondeson L, Lindholm K, Thorstenson S: Benign dermal eccrine cylindroma. A pitfall in the cytologic diagnosis of adenoid cystic carcinoma. *Acta Cytol* 1983;27:326-328.
136. Eneroth CM, Zajicek J: Aspiration biopsy of salivary gland tumors. III. Morphologic studies on smears and histologic sections from 368 mixed tumors. *Acta Cytol* 1966;10:440-454.
137. Heintz PW, Schmidt WA, Pommier RF, et al.: Submandibular gland carcinoma ex pleomorphic adenoma. Report of a case with cytologic features and diagnostic pitfalls. *Acta Cytol* 1998;42:1431-1436.
138. Wenig BM, Hitchcock CL, Ellis GL, Gnepp DR: Metastasizing mixed tumor of salivary glands. A clinicopathologic and flow cytometric analysis. *Am J Surg Pathol* 1992;16:845-858.
139. Pitman MB, Thor AD, Goodman ML, Rosenberg AE: Benign metastasizing pleomorphic adenoma of salivary gland: diagnosis of bone lesions by fine-needle aspiration biopsy. *Diagn Cytopathol* 1992;8:384-387.
140. Granger JK, Houn HY: Malignant mixed tumor (carcinosarcoma) of parotid gland diagnosed by fine-needle aspiration biopsy. *Diagn Cytopathol* 1991;7:427-432.
141. Kim T, Yoon GS, Kim O, Gong G: Fine needle aspiration diagnosis of malignant mixed tumor (carcinosarcoma) arising in pleomorphic adenoma of the salivary gland. A case report. *Acta Cytol* 1998;42:1027-1031.
142. Sironi M, Isimbaldi G, Claren R, et al.: Carcinosarcoma of the parotid gland: Cytological, clinicopathological and immunohistochemical study of a case. *Pathol Res Pract* 2000;196:511-517.
143. Delgado R, Klimstra D, Albores-Saavedra J: Low grade salivary duct carcinoma. A distinctive variant with a low grade histology and a predominant intraductal growth pattern. *Cancer* 1996;78:958-967.
144. Brandwein-Gensler M, Hille J, Wang BY, et al.: Low-grade salivary duct carcinoma: Description of 16 cases. *Am J Surg Pathol* 2004;28(8):1040-1044.
145. Elsheikh TM, Bernacki EG, Pisharodi L: Fine-needle aspiration cytology of salivary duct carcinoma. *Diagn Cytopathol* 1994;11:47-51.
146. Fyrat P, Cramer H, Feczko JD, et al.: Fine-needle aspiration biopsy of salivary duct carcinoma: Report of five cases. *Diagn Cytopathol* 1997;16:526-530.
147. Gal R, Strauss M, Zohar Y, Kessler E: Salivary duct carcinoma of the parotid gland. Cytologic and histopathologic study. *Acta Cytol* 1985;29:454-456.
148. Garcia-Bonafe M, Catala I, Tarragona J, Tallada N: Cytologic diagnosis of salivary duct carcinoma: a review of seven cases. *Diagn Cytopathol* 1998;19:120-123.
149. Gilcrease MZ, Guzman-Paz M, Froberg K, Pambuccian S: Salivary duct carcinoma. Is a specific diagnosis possible by fine needle aspiration cytology? *Acta Cytol* 1998;42:1389-1396.
150. Khurana KK, Pitman MB, Powers CN, et al.: Diagnostic pitfalls of aspiration cytology of salivary duct carcinoma. *Cancer* 1997;81:373-378.
151. Kljanienco J, Vielh P: Cytologic characteristics and histomorphologic correlations of 21 salivary duct carcinomas. *Diagn Cytopathol* 1998;19:333-337.
152. Castle JT, Thompson LD, Frommelt RA, et al.: Polymorphous low grade adenocarcinoma: A clinicopathologic study of 164 cases. *Cancer* 1999;86:207-219.
153. Evans HL, Luna MA: Polymorphous low-grade adenocarcinoma: a study of 40 cases with long-term follow up and an evaluation of the importance of papillary areas. *Am J Surg Pathol* 2000;24:1319-1328.
154. Gibbons D, Saboorian MH, Vuitch F, et al.: Fine-needle aspiration findings in patients with polymorphous low grade adenocarcinoma of the salivary glands. *Cancer* 1999;87:31-36.

155. Kljianienko J, Vielh P: Salivary carcinomas with papillae: cytology and histology analysis of polymorphous low-grade adenocarcinoma and papillary cystadenocarcinoma. *Diagn Cytopathol* 1998;19:244-249.
156. Watanabe K, Ono N, Saito K, et al.: Fine-needle aspiration cytology of polymorphous low-grade adenocarcinoma of the tongue. *Diagn Cytopathol* 1999;20:167-169.
157. Ellis GL, Wiscovitch JG: Basal cell adenocarcinomas of the major salivary glands. *Oral Surg Oral Med Oral Pathol* 1990;69:461-469.
158. Murty GE, Welch AR, Soames JV: Basal cell adenocarcinoma of the parotid gland. *J Laryngol Otol* 1990;104:150-151.
159. Fonseca I, Soares J: Epithelial-myoepithelial carcinoma of the salivary glands. A study of 22 cases. *Virchows Arch A Pathol Anat Histopathol* 1993;422:389-396.
160. Arora VK, Misra K, Bhatia A: Cytomorphologic features of the rare epithelial-myoepithelial carcinoma of the salivary gland. *Acta Cytol* 1990;34:239-242.
161. Carrillo R, Poblet E, Rocamora A, Rodriguez-Peralto JL: Epithelial-myoepithelial carcinoma of the salivary gland. Fine needle aspiration cytologic findings. *Acta Cytol* 1990;34:243-247.
162. Miliauskas JR, Orell SR: Fine-needle aspiration cytological findings in five cases of epithelial-myoepithelial carcinoma of salivary glands. *Diagn Cytopathol* 2003;28(3):163-167.
163. Layfield LJ, Glasgow BJ: Aspiration cytology of clear-cell lesions of the parotid gland: Morphologic features and differential diagnosis. *Diagn Cytopathol* 1993;9:705-711.
164. Milchgrub S, Gnepp DR, Vuitch F, et al.: Hyalinizing clear cell carcinoma of salivary gland. *Am J Surg Pathol* 1994;18:74-82.
165. Milchgrub S, Vuitch F, Saboorian MH, et al.: Hyalinizing clear-cell carcinoma of salivary glands in fine-needle aspiration. *Diagn Cytopathol* 2000;23:333-337.
166. Batsakis JG: Clear cell tumors of salivary glands. *Ann Otol Rhinol Laryngol* 1980;89:196-197.
167. Eveson JW: Troublesome tumours 2: borderline tumours of salivary glands. *J Clin Pathol* 1992;45:369-377.
168. Galanis E, Frytak S, Lloyd RV: Extrapulmonary small cell carcinoma. *Cancer* 1997;79:1729-1736.
169. Gnepp DR, Wick MR: Small cell carcinoma of the major salivary glands. An immunohistochemical study. *Cancer* 1990;66:185-192.
170. Gnepp DR, Corio RL, Brannon RB: Small cell carcinoma of the major salivary glands. *Cancer* 1986;58:705-714.
171. Hui KK, Luna MA, Batsakis JG, et al.: Undifferentiated carcinomas of the major salivary glands. *Oral Surg Oral Med Oral Pathol* 1990;69:76-83.
172. Ibrahim NB, Briggs JC, Corbishley CM: Extrapulmonary oat cell carcinoma. *Cancer* 1984;54:1645-1661.
173. Cameron WR, Johansson L, Tennvall J: Small cell carcinoma of the parotid. Fine needle aspiration and immunochemical findings in a case. *Acta Cytol* 1990;34:837-841.
174. Henke AC, Cooley ML, Hughes JH, Timmerman TG: Fine-needle aspiration cytology of small-cell carcinoma of the parotid. *Diagn Cytopathol* 2001;25:126-129.
175. Chan JK, Suster S, Wenig BM, et al.: Cytokeratin 20 immunoreactivity distinguishes Merkel cell (primary cutaneous neuroendocrine) carcinomas and salivary gland small cell carcinomas from small cell carcinomas of various sites. *Am J Surg Pathol* 1997;21:226-234.
176. Gunhan O, Celasun B, Safali M, et al.: Fine needle aspiration cytology of malignant lymphoepithelial lesion of the salivary gland. A report of two cases. *Acta Cytol* 1994;38:751-754.
177. Safneck JR, Ravinsky E, Yazdi HM, et al.: Fine needle aspiration biopsy findings in lymphoepithelial carcinoma of salivary gland. *Acta Cytol* 1997;41:1023-1030.
178. Thompson MB, Nestok BR, Gluckman JL: Fine needle aspiration cytology of lymphoepithelioma-like carcinoma of the parotid gland. A case report. *Acta Cytol* 1994;38:782-786.
179. Batsakis JG, el-Naggar AK, Luna MA: "Adenocarcinoma, not otherwise specified": A diminishing group of salivary carcinomas. *Ann Otol Rhinol Laryngol* 1992;101:102-104.
180. Chhieng DC, Cangiarella GF, Cohen JM: Fine-needle aspiration cytology of lymphoproliferative lesions involving the major salivary glands. *Am J Clin Pathol* 2000;113:563-571.
181. Hyjek E, Smith WJ, Isaacson PG: Primary B-cell lymphoma of salivary glands and its relationship to myoepithelial sialadenitis. *Hum Pathol* 1988;19:766-776.
182. Quintana PG, Kapadia SB, Bahler DW, et al.: Salivary gland lymphoid infiltrates associated with lymphoepithelial lesions: A clinicopathologic, immunophenotypic, and genotypic study. *Hum Pathol* 1997;28:850-861.
183. Cha I, Long SR, Ljung BM, Miller TR: Low-grade lymphoma of mucosa-associated tissue in the parotid gland: A case report of fine-needle aspiration cytology diagnosis using flow cytometric immunophenotyping. *Diagn Cytopathol* 1997;16:345-349.
184. Young NA, Al-Saleem T: Diagnosis of lymphoma by fine-needle aspiration cytology using the revised European-American classification of lymphoid neoplasms. *Cancer* 1999;87:325-345.
185. Lussier C, Kljianienko J, Vielh P: Fine-needle aspiration of metastatic nonlymphomatous tumors to the major salivary glands: A clinicopathologic study of 40 cases cytologically diagnosed and histologically correlated. *Cancer* 2000;90:350-356.
186. Zhang C, Cohen JM, Cangiarella JF, et al.: Fine-needle aspiration of secondary neoplasms involving the salivary glands. A report of 36 cases. *Am J Clin Pathol* 2000;113:21-28.
187. Hilborne LH, Glasgow BJ, Layfield LJ: Fine-needle aspiration cytology of juvenile hemangioma of the parotid gland: A case report. *Diagn Cytopathol* 1987;3:152-155.
188. Layfield LJ, Mooney EE, Dodd LG: Not by blood alone: diagnosis of hemangiomas by fine-needle aspiration. *Diagn Cytopathol* 1998;250-254.

Lymph Nodes

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TECHNICAL ASPECTS

REPORTING TERMINOLOGY AND ACCURACY

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Flow Cytometry
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NON-NEOPLASTIC LESIONS

Reactive Hyperplasia
Miscellaneous Benign Lymphadenopathies
Inflammatory and Infectious Conditions
Sarcoidosis
Bacterial and Fungal Lymphadenitis
Cat Scratch Disease
Mycobacterial Lymphadenitis

Rosai-Dorfman Disease (Sinus Histiocytosis with Massive Lymphadenopathy)
Kikuchi Lymphadenitis
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NEOPLASMS

Hodgkin Lymphoma
Non-Hodgkin Lymphoma
Lymphomas of Small Cells
Lymphomas of Large Cells
Nonlymphoid Neoplasms
Carcinomas
Malignant Melanoma
Seminoma and Germioma
Sarcomas

Enlarged lymph nodes are a prime target for fine-needle aspiration (FNA). In an adult, lymphadenopathy is an immediate source of concern, and unless the cause is evident, the enlarged node is usually aspirated. In children, lymphadenopathy is common and usually the result of reactive hyperplasia; for this reason, it is often watched and not aspirated. Nevertheless, FNA is readily applicable to children also, particularly if lymphadenopathy persists.

Indications for lymph node fine-needle aspiration:

- confirm a clinical impression of reactive hyperplasia
- diagnose a suspected infection
- diagnose a suspected malignancy
 - Hodgkin lymphoma
 - non-Hodgkin lymphoma
 - metastatic tumor of unknown primary
- document a metastasis in a patient with a known malignancy
- confirm transformation of a known lymphoma to one of higher grade

FNA is accepted by most patients as a non-invasive method for evaluating lymphadenopathy. It has advantages compared to surgical excision and preserves lymph node architecture should an excision be necessary.

Advantages of lymph node fine-needle aspiration:

- rapid turnaround time
- low cost
- easily provides cells for immunophenotyping and molecular diagnostic tests
- less morbidity

FNA is particularly useful in patients with deep-seated lymphadenopathy (e.g., mediastinal, retroperitoneal, abdominal), for which surgical intervention carries the risk of significant morbidity. Even with superficial (e.g., cervical) lymphadenopathy, using FNA avoids uncommon but serious morbidity associated with lymph node excision, like accessory spinal nerve injury.¹

Whether FNA should be employed in a patient suspected of having non-Hodgkin lymphoma (NHL) remains controversial. In some individuals FNA is ideal for this indication, particularly those with rapidly progressive disease, an oncologic emergency (e.g., spinal cord compression, airway compromise, superior vena cava syndrome), deep or surgically inaccessible nodes, advanced age, or comorbid clinical conditions that preclude surgical biopsy or excision.² When excisional biopsy is less problematic, a larger amount of tissue might be desired at academic institutions for correlative research studies involving emerging technologies like proteomics and genomics.

Limitations of lymph node fine-needle aspiration:

- sampling error
 - small or deep-seated lymph node
 - nodal fibrosis
 - excessive necrosis or inflammation
 - partial involvement of lymph node by the lesion
- important architectural or vascular patterns are lost in some entities:
 - progressively transformed germinal centers
 - human immunodeficiency virus (HIV) lymphadenopathy
 - vascular transformation of lymph node sinuses
 - toxoplasma lymphadenitis
 - Castleman disease
 - nodular lymphocyte predominant Hodgkin lymphoma
 - diffuse large B-cell lymphoma arising in follicular lymphoma

TECHNICAL ASPECTS

The principle guiding the cytopathologist is the same as that guiding the surgical pathologist: the integration of clinical information, light microscopic analysis, and results of ancillary studies into a final diagnosis. The full value of FNA is only achieved with this integrated approach.³ The cytopathologist who performs the FNA, in fact, has a great opportunity (and responsibility) to incorporate clinical history and physical findings into the final diagnosis.⁴

FNAs are performed by pathologists and clinicians. Pathologists have an advantage in that they can perform an on-site adequacy assessment and repeat the procedure until adequate material is obtained. The on-site assessment performs another vital function: triage of the specimen. If the initial smears raise the possibility of lymphoma, the needle rinse can be submitted for special studies such as immunocytochemistry, flow cytometry, cytogenetics, and molecular cytogenetics. Similarly, if there is evidence of an infection (neutrophils, granulomas, necrosis, visible organisms), a portion of the specimen can be submitted for microbiologic culture.

Most pathologists do not use local anesthesia to perform a superficial FNA, but some do employ a topical cream or lidocaine injection. If anesthetic is injected, it should be above, rather than on or into, the node. Betadine cleansing of the skin, sterile towels, and expensive prepackaged trays are unnecessary; alcohol swabs are sufficient. In general, at least three separate passes (needle puncture with aspiration) are made with a 22-, 23-, or 25-gauge sterile needle. (Radiologists performing aspirations of deep-seated lymph nodes sometimes use the larger

(but still “fine”) 20-gauge needle because smaller needles bend too easily and may not reach their target.) The procedure is performed either with the needle attached to a syringe so that a vacuum is applied or with the needle alone. A syringe holder assists greatly in allowing one to fix the node in place with one hand and pull back the syringe for vacuum suction with the other. The needle-only technique is useful for small (<1 cm) lymph nodes, or those that are difficult to immobilize, but one is generally able to procure more cells when using a syringe with vacuum suction. Care in the preparation of smears is necessary; lymphocytes are fragile and easily crushed if too much pressure is applied during the making of smears.

Most slides are air-dried and stained with a Romanowsky (Wright-Giemsa, May-Grünwald-Giemsa, Diff-Quik®, Hemacolor®) type of stain. A smaller percentage is stained with a modified Papanicolaou stain. Most pathologists rely heavily on the Romanowsky stain because it highlights cytoplasmic details of lymphoid cells and lymphoglandular bodies. Furthermore, because it is the same stain used for bone marrow aspirates and peripheral blood, it makes for better comparison with other hematologic specimens. The Papanicolaou stain highlights nuclear details (chromatin texture, nucleoli, convolutions, and knobs) and the orangeophilia of a metastatic squamous cell carcinoma. Because these stains complement each other, both should be used whenever possible. Every needle pass is rinsed in a balanced salt solution after material is expelled onto slides; the needle rinse is useful for ancillary studies (flow cytometry, cytopins, paraffin-embedded cell blocks) as needed. RPMI-1640 cell culture medium is recommended by some practitioners, but normal saline is also acceptable.

The major relative contraindication is a severe coagulation disorder; however, if one proceeds with an FNA, pressure can be applied to a superficial node after aspiration to prevent a hematoma, and appropriate blood products can be given in the case of a deep-seated node aspiration. Complications of lymph node FNA are rare. The most common is a hematoma. Data are sparse on the frequency of a hematoma, but experience suggests that it occurs in <1% of cases. Tissue artifacts attributable to FNA (focal hemorrhage with organization; segmental or total infarction) are seen in only 4% of excised lymph nodes⁵ and rarely preclude histologic analysis.⁶ A pneumothorax can result from aspiration of a deep axillary, low cervical, or supraclavicular lymph node if the pleural space is entered inadvertently.

REPORTING TERMINOLOGY AND ACCURACY

Terminology for reporting lymph node aspiration results is similar to that used for most cytologic specimens. Results are classified into several broad categories:

non-diagnostic (unsatisfactory), negative, atypical, suspicious or positive for malignant cells. The frequency of nondiagnostic (unsatisfactory) results ranges from 5% to 15% of cases⁷⁻¹⁰ and is site dependent and size dependent. For example, it is more difficult to obtain adequate material from a small, deep axillary node than a large cervical lymph node.

A negative result does not exclude malignancy. For this reason, an explanatory note is helpful, such as “Clinical correlation is advised to ensure that the sample is representative. If clinical suspicion of malignancy persists, additional tissue sampling should be considered.”

FNA of lymph nodes has high sensitivity and specificity in the distinction between a benign and malignant lesion. Accuracy estimates for lymph node FNA vary because of local variations in technique and referral patterns, but most investigators report over 90% accuracy in the diagnosis of metastatic tumor to lymph nodes, and a positive predictive value of almost 100%.^{10,11} Similarly, the accuracy of a diagnosis of Hodgkin lymphoma is high,^{12,13} with a positive predictive value over 90%.¹⁴

Some oncologists question the use of FNA for the diagnosis of NHL. One study reported no clinical benefit from FNA,¹⁵ yet in fewer than 50% of the study cases was immunophenotyping (IP) performed and in only one third did diagnoses conform to the World Health Organization's (WHO) lymphoma classification. The importance of using modern practice guidelines when evaluating an FNA for the possibility of lymphoma can not be overemphasized. At a minimum, the cytologist must have a working knowledge of the WHO classification¹⁶; some of the sample must be allocated for IP; and a close interaction with hematopathologists must be fostered. Under such circumstances, the accuracy of FNA for the diagnosis of NHL is quite good. Most studies published in the last decade show a sensitivity for recognizing NHL that is higher than 80%,^{3,17,18} with specificity greater than 90%. Accuracy is even higher when FNA is performed for recurrent, as opposed to newly diagnosed, NHL.

Regarding subclassification, some lymphomas are easier to subtype precisely than others. IP, either by flow cytometry^{19,20} (preferred) or immunocytochemistry, plays a vital role in the diagnosis and subtyping of lymphomas; without it, the accuracy of FNA is severely diminished. Lymphomas that have characteristic immunophenotypes, like small lymphocytic lymphoma (SLL), mantle cell lymphoma (MCL), and lymphoblastic lymphoma, are easier to recognize than those that do not.¹² The prevalence of different subtypes in the pediatric and adult populations explains in part why FNA is highly accurate in pediatric lymphomas,^{21,22} and less so in adult NHL. Differentiating the so-called “small cell lymphomas” from each other and from reactive hyperplasia by

morphology alone is a common dilemma.⁷⁻⁹ Molecular techniques (discussed herein) are playing an increasingly important role as adjuncts to FNA, particularly in the diagnosis and subtyping of NHL.

ANCILLARY STUDIES

When evaluating a lymph node FNA for a possible lymphoproliferative disorder, a variety of specialized studies are indispensable. These require additional effort and increase the turnaround time of the case, but the extra effort and time are often rewarded by precise diagnosis and classification of the neoplastic process.

Ancillary studies help to:

- distinguish lymphoid from nonlymphoid lesions
- distinguish NHL from reactive lesions by confirming clonality
- subclassify a lymphoma

Clonality in B-cell lymphomas is documented by demonstrating light chain restriction; a clonal expansion of B cells that preferentially express either κ or λ immunoglobulin light chains. Clonal B-cell proliferations identified by flow cytometry almost never occur in reactive lymph nodes, but exceptions have been documented.²³ Therefore, it cannot be overemphasized that all aspects of the case—morphology, IP results, and clinical features—must be taken into consideration before issuing a diagnosis of lymphoma.

Lymphomas are subclassified into biologically meaningful subtypes by their expression of characteristic markers—terminal deoxynucleotidyl transferase (Tdt) in lymphoblastic lymphoma, anaplastic lymphoma kinase (ALK) protein in anaplastic large cell lymphoma, differential expression of CD5, CD10, bcl-1, and CD23 in the small cell lymphomas. Because aberrant results occasionally occur with every marker, it is advisable to use a panel of antibodies (the result of one is a double-check against the others) rather than any one in isolation.

IP, to determine B-cell clonality and document antigen expression, can be performed by immunocytochemistry, usually on cytocentrifuge preparations,^{24,25} or by flow cytometry.^{8,12,20,26-28} Each method has its advantages (Table 11.1).

Advantages of flow cytometry:

- rapid turnaround (2 hours)
- quantitative (number of cells and intensity)
- multiple stains per cell
- superior detection of small monoclonal populations

TABLE 11.1 COMPARISON OF FLOW CYTOMETRY AND IMMUNOCYTOCHEMISTRY FOR THE ANALYSIS OF LYMPHOID MARKERS

	Flow Cytometry	Immunocytochemistry
Morphology	lost	preserved
Sensitivity	high	lower
Cells required	many	fewer
Detection of small monoclonal population	good	fair to poor
Multiple labeling	easy	laborious
Hodgkin lymphoma	useless	useful
Turnaround time	2 hours	24–48 hours

Advantages of immunocytochemistry:

- morphology is preserved
- fewer cells needed
- useful in detecting Reed-Sternberg cells

To fully characterize a lymphoma, between 12 and 15 antibodies may be needed,²⁹ but often fewer are sufficient. If the sample is sparsely cellular, the cytologist can assist the flow cytometry or immunocytochemistry laboratory by using cell morphology to select an appropriate, more limited panel of antibodies.

Flow Cytometry

The routine application of flow cytometry (FCM) has markedly improved the diagnostic accuracy of FNA.^{30,31} In a flow cytometer, a liquid suspension of single cells

is made to flow in single file through a laser beam (Fig. 11.1). As each cell traverses the beam, it scatters light in different directions.

The amount of light scattered in different directions gives useful information about the cell:

- forward scatter: proportional to cell size
- side scatter: proportional to cytoplasmic complexity (often as a result of granularity)

Using forward and side scatter alone, a flow cytometer easily distinguishes normal lymphocytes, monocytes, and polymorphonuclear leukocytes.

Along with these “natural” qualities, lymphoid cells are characterized by their expression of different lymphocyte markers, which are identified using antibodies conjugated to a fluorescent molecule (fluorochrome). When excited by a laser beam, a fluorochrome emits a pulse of light of a specific wavelength (color). The intensity is proportional to the amount of fluorochrome-conjugated antibody attached to that cell. For example, if we incubate a suspension of cells with fluorochrome-conjugated antibodies to CD19, a marker of B cells, each B cell will emit a bright pulse of light, but T cells, which lack CD19, will show only background fluorescence.

Commonly used fluorochromes that emit pulses of different-colored light when excited by a laser beam:

- fluorescein isothiocyanate (FITC)
- phycoerythrin (PE)
- peridinin chlorophyll protein (PerCP)

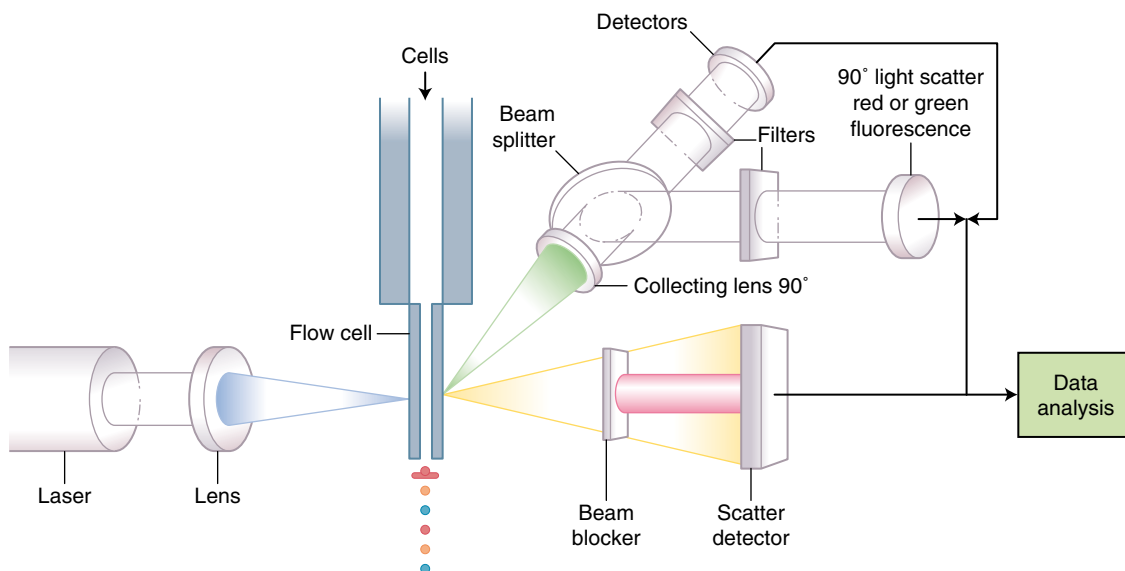


Figure 11.1 Schematic diagram of a flow cytometer. Cells are examined as they flow in single file through a laser beam, directed at right angles to the flow stream. Light scatter and fluorescence of each cell are measured by detectors and analyzed.

With two fluorochromes that have different emission spectra, one can incubate cells with two antibodies, each conjugated with a different fluorochrome. The intensity of staining of cells for the two antibodies is plotted as a two-dimensional dot-plot (scattergram).

Advantages of using multicolor cytometry:

- fewer tubes needed
- multiple antigens can be examined on each cell

Four-color FCM is standard, but many laboratories use more fluorochromes for comprehensive IP. Being able to analyze multiple antigens per cell improves the sensitivity in the detection of clonal B cells. For example, a small proportion of CD5-positive, κ -restricted B cells, in a background of many reactive polytypic B cells, can be detected by four-color FCM when the sample is simultaneously labeled with four antibodies (CD19, CD5, κ , λ), but will go undetected with fewer antibodies. For most clinical applications, four-color FCM is adequate. For a full lymphoma panel, 10 aliquots of 1×10^5 cells each, or a total of 1×10^6 cells, is usually sufficient.

Immunocytochemistry

With immunocytochemistry, the presence of cell markers is probed on cells attached to a glass slide. Immunohistochemistry can be performed on formalin-fixed, paraffin-embedded sections from cell block preparations, but some antigens, notably κ and λ immunoglobulin light chains, are difficult to evaluate in fixed specimens. For this reason, unfixed (air-dried) preparations are best for most lymph node FNAs when lymphoma is a consideration. Commonly, slides are prepared by cytocentrifugation onto a 6-mm circle on a glass slide; the limited area to be evaluated conserves antibodies and facilitates interpretation of results. Some of the most commonly used markers are listed in Table 11.2. Immunostaining methods vary slightly from one laboratory to another.^{24,25}

Molecular Genetic Studies

Just as immunocytochemistry localizes specific proteins in cells, molecular genetic studies identify specific DNA sequences. They are especially helpful when IP methods are inconclusive in establishing clonality or cell lineage. For example, T-cell clonality can only be established by these methods.

Molecular techniques available:

- polymerase chain reaction
- fluorescence in situ hybridization (FISH)
- gene expression profiling

TABLE 11.2 USEFUL IMMUNOCYTOCHEMICAL MARKERS FOR THE DIAGNOSIS OF LYMPHOPROLIFERATIVE DISORDERS

CD3	T cells
CD5	T cells, coexpressed in some B-cell lymphomas (SLL and MCL)
CD19	B cells
CD20	B cells
CD45	most lymphoid cells
kappa (κ)	B-cell immunoglobulin light chain (assessing clonality)
lambda (λ)	B-cell immunoglobulin light chain (assessing clonality)
cyclin D1	mantle cell lymphoma
CD15 (LeuM1)	Reed–Sternberg cells (except nodular lymphocyte predominant Hodgkin lymphoma)
CD30 (Ki-1)	Reed–Sternberg cells, anaplastic large cell lymphoma
ALK	anaplastic large cell lymphoma
Tdt	lymphoblastic lymphoma

ALK, anaplastic lymphoma kinase; MCL, mantle cell lymphoma; SLL, small lymphocytic lymphoma; Tdt, terminal deoxynucleotidyl transferase.

The *polymerase chain reaction (PCR)* amplifies tiny amounts of a defined region of DNA, provided that sequences surrounding the region of interest are known. The technique requires repeated cycles of denaturation and DNA synthesis using oligonucleotide primers, a heat-resistant DNA polymerase, and the four nucleotides. The oligonucleotide primers are synthesized to complement DNA sequences flanking the region of interest. Typically, 20 to 60 cycles of denaturation and synthesis over several hours result in a tremendous amplification of a homogeneous set of the DNA sequence of interest. Because of its extraordinary sensitivity, however, it is prone to false-positive results; rigorous controls and critical review of the results are vital. PCR is a useful test for immunoglobulin and T-cell receptor rearrangements as markers of clonality.^{6,28,32-36} PCR for clonality is redundant for B-cell neoplasms that are shown to be monoclonal by IP, but it is useful in select circumstances (e.g., detecting T-cell clonality).^{32,37} PCR is fast (1 to 2 days), does not require radioactive probes, and can be performed on formalin-fixed cell-blocks. It can be used as a confirmatory test when FCM suggests a T-cell neoplasm, when cells fail to survive FCM processing, or when unfixed cells are unavailable for FCM.

PCR can be used to detect specific breakpoints in lymphomas. This is especially helpful if (1) the patient has a known lymphoma with a characteristic translocation and the FNA is carried out to rule out recurrence or (2) in the case of a primary diagnosis of lymphoma, if the morphology suggests a specific subtype of lymphoma with a characteristic translocation. Because a

translocation characteristic of a particular type of lymphoma can occur at different breakpoints in different patients, PCR may have low sensitivity if only one breakpoint is probed.

With *fluorescence in situ hybridization (FISH)*, DNA probes for specific chromosomal regions, labeled with a fluorochrome, such as rhodamine, are hybridized with intact nuclei. Smears, cytocentrifuge preparations, and thinlayer slides are ideal substrates because the intact cells on cytologic preparations allow optimal signal detection. DNA probes are particularly useful for demonstrating translocations,³⁸ deletions, and gene amplifications (e.g., trisomies). Some practitioners recommend that cells be allocated for PCR and FISH, in case they are needed, in addition to FCM, in every case of suspected lymphoma.³⁹

Gene expression profiling using microarray analysis is gradually finding a role in the cytologic diagnosis of lymphoma. Aspirates of suspected NHL can be rinsed in a ribonucleic acid (RNA) stabilization reagent. Gene expression profiling is performed by hybridizing RNA to gene chips.⁴⁰ Among other potential applications, gene expression profiles can be used to distinguish among different prognostic subtypes of diffuse large B-cell lymphoma (DLBL).⁴¹

NON-NEOPLASTIC LESIONS

The normal microanatomy of a lymph node consists of a cortex, paracortex, and medulla, each with a different mix of cells that include small B and T lymphocytes, follicular center cells (centrocytes and centroblasts), plasma cells, plasmacytoid lymphocytes, immunoblasts, follicular dendritic cells, interdigitating reticulum cells, tingible-body macrophages, endothelial cells, mast cells, and eosinophils. Some of these cells are morphologically similar (but immunologically different), whereas others show distinctive morphologic features. Truly normal lymph nodes are rarely aspirated because they are too small to produce a palpable mass. Most normal, but enlarged lymph nodes are examples of hyperplasia, in which various cellular components of a normal node are increased.

Two consistent findings in aspirated lymphoid tissue:

- a dispersed cell pattern
- lymphoglandular bodies

A dispersed, isolated cell pattern is typical of lymphoid cells, but there are exceptions. Smear thickness, clotting, and suboptimal spreading technique may cause clustering of lymphocytes. Aggregates of lymphocytes adherent to follicular dendritic cells occur in follicular hyperplasia and follicular lymphoma,^{42,43} where they

are known as dendritic-lymphocytic aggregates. Unlike most lymphomas, anaplastic large cell lymphoma shows cell cohesion. Conversely, some nonlymphoid tumors (e.g., melanoma) lack cohesion and mimic malignant lymphoma.

Lymphoid globules are fragments of lymphoid cell cytoplasm given the fanciful (and imprecise) name “lymphoglandular bodies.”⁴⁴ They are usually numerous in virtually all lymphoid proliferations, both benign and malignant. In Romanowsky-stained smears they are a pale blue or blue-gray, and may contain tiny vacuoles; they are much more difficult to see in Papanicolaou-stained smears. Rarely, they are seen in a small number of nonlymphoid lesions such as small cell neuroendocrine carcinoma,⁴⁵ but, in general, the absence of lymphoglandular bodies makes it unlikely that the cells are of lymphoid origin.

Reactive Hyperplasia

Reactive lymphoid hyperplasia is a common nonspecific form of lymphadenopathy resulting from a variety of causes in the pediatric and young adult population; it is less frequent in older adults. Most cases of reactive lymphoid hyperplasia are examples of follicular rather than paracortical hyperplasia. The excursions of an aspirating needle capture cells from the expanded cortex, paracortex, or medulla that, randomly commingled within the barrel of the needle, are then expelled onto the slide as a rich, heterogeneous mixture.

CYTOMORPHOLOGY OF REACTIVE HYPERPLASIA:

- polymorphous population
- small lymphocytes
- plasmacytoid lymphocytes
- centrocytes
- centroblasts
- immunoblasts
- tingible-body macrophages
- dendritic-lymphocytic aggregates
- capillaries, eosinophils, mast cells

There is great variety in the cell types encountered (Fig. 11.2). *Small lymphocytes* with round nuclei and coarsely textured chromatin predominate, but they are admixed with other cells. *Centrocytes* are intermediate-sized lymphocytes with irregular or cleaved nuclei, inconspicuous nucleoli, and scant cytoplasm. *Centroblasts* are large cells with round, vesicular nuclei, one to three peripheral nucleoli, and a narrow rim of basophilic cytoplasm. *Immunoblasts* are large cells with fine, “open” chromatin, one prominent, centrally located nucleolus, and moderate to abundant, pale to basophilic cytoplasm. Some have a perinuclear clear zone.

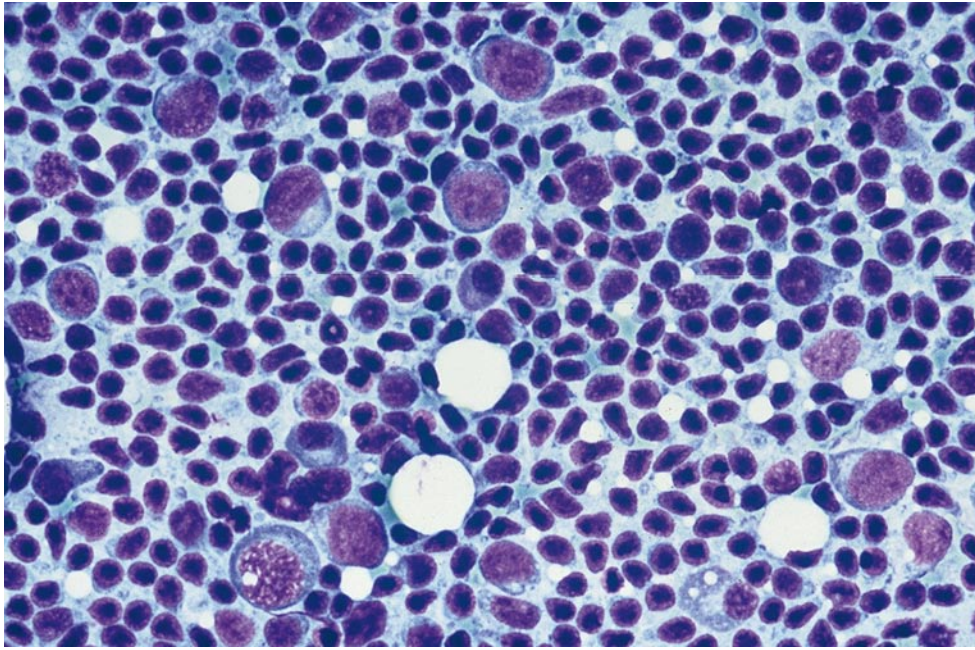


Figure 11.2 Reactive lymphoid hyperplasia. Immunoblasts and plasmacytoid lymphocytes are interspersed throughout a smear dominated by small round lymphocytes, creating a polymorphous cell picture (Romanowsky stain).

Tingible-body macrophages are large phagocytic cells with a spherical nucleus, finely granular chromatin, a small distinct nucleolus, and voluminous debris-laden cytoplasm. *Dendritic cells* are also large, with pale oval nuclei, indistinct nucleoli, and cytoplasmic processes; they may be binucleated or multinucleated.

Dendritic-lymphocytic aggregates (also known as follicular center fragments when tingible-body macrophages and capillaries are present), are clusters of dendritic cells, small lymphocytes, centrocytes, and centroblasts⁴² (Figs. 11.3, 11.4). Distinguishing between fragments that do or do not contain tingible-body macrophages and capillaries does not appear to have diagnostic value. These aggregates break the rule that lymphoid

lesions lack cell cohesion; they are seen whenever there are germinal centers (follicles).

Because little stroma exists to retain lymphoid cells during aspiration, large numbers of cells are obtained, resulting in highly cellular smears. Thus, high cellularity in a lymph node FNA does not correlate with malignancy. When reactive lymphoid hyperplasia is subdivided into early, mid, and late phases, small round lymphocytes predominate in all stages, with variations in the percentage of other lymphoid cells.^{4,46} In most examples of reactive lymphoid hyperplasia, small lymphocytes vastly outnumber centroblasts and centrocytes, which are themselves more numerous than immunoblasts, plasmacytoid lymphocytes, and tingible-body macrophages.

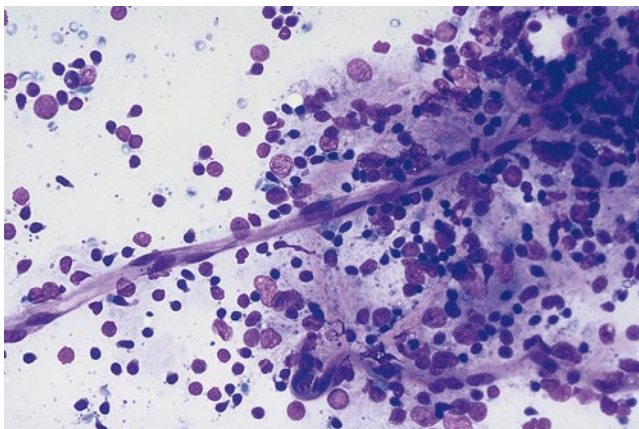


Figure 11.3 Reactive lymphoid hyperplasia. A capillary emanates diagonally from this follicular center fragment (dendritic-lymphocytic aggregate), which contains a mixture of dendritic cells and a heterogeneous lymphocyte population (Romanowsky stain).

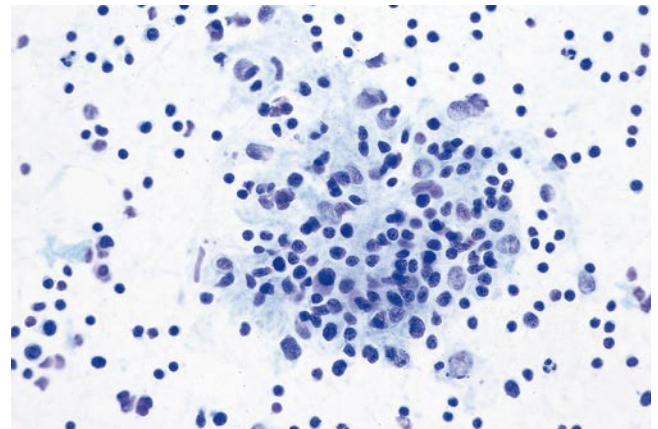


Figure 11.4 Reactive lymphoid hyperplasia. This dendritic-lymphocytic aggregate is a loose collection of small round lymphocytes and dendritic cells. The latter have pale nuclei with delicate cytoplasmic extensions (Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF REACTIVE HYPERPLASIA:

- NHL, especially:
 - follicular lymphoma
 - marginal zone or mucosa-associated lymphoid tissue (MALT) lymphoma
 - T-cell lymphoma
 - T-cell-rich large B-cell lymphoma
- Hodgkin lymphomas
- partial node involvement by a neoplasm or non-neoplastic lesion
- post-transplant lymphoproliferative disorder
- other benign lymphadenopathies

The major challenge is to exclude a lymphoma. Because architectural pattern is not available, cell size, smear composition, and immunophenotypic studies are used as discriminators. The most helpful morphologic feature is the heterogeneity of the lymphocytes; in NHL the range of lymphoid forms is usually narrower. There are exceptions; Hodgkin lymphoma, follicular lymphoma, marginal zone/MALT lymphoma, some T-cell lymphomas, and especially T-cell-rich large B-cell lymphoma have a heterogeneous appearance that mimics reactive lymphoid hyperplasia. The distinction in such cases relies on identifying rare atypical forms (e.g., Reed-Sternberg [RS] cells or lymphocytic and histiocytic [L & H] cells in Hodgkin lymphoma) or IP, which should be used whenever a lymph node, either clinically or morphologically, is suspicious for NHL.

In the case of partial lymph node involvement by malignancy, sampling error can result in a false impression of reactive lymphoid hyperplasia. In such instances, if the node is clinically suspicious, a negative result should not deter a followup excisional biopsy or repeat FNA. Although often mentioned as a potential pitfall, sampling error is uncommon in actual practice because varying the angle of entry of the aspirating needle with each pass usually adequately samples the majority of enlarged nodes.

In a patient who has received a solid organ or bone marrow transplant, the possibility of a post-transplant lymphoproliferative disorder should be considered. In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA (EBER) can be helpful in this setting.⁴⁷

Miscellaneous Benign Lymphadenopathies

Some lymphadenopathies have distinct architectural features that allow for a specific diagnosis in tissue sections. These features are not readily apparent in smears, and therefore, in most circumstances, these lymphadenopathies cannot be reliably distinguished from reactive lymphoid hyperplasia by FNA.

Lymphadenopathies that cannot be reliably distinguished from reactive lymphoid hyperplasia by fine-needle aspiration:

- Castleman disease
- toxoplasma lymphadenitis
- human immunodeficiency virus (HIV)-associated lymphadenopathy
- progressively transformed germinal centers
- dermatopathic lymphadenopathy

Castleman disease (CD) is a form of lymph node hyperplasia and is divided into hyaline-vascular and plasma cell types. The most common sites are the mediastinum and cervical lymph nodes. In the hyaline-vascular type, tissue sections reveal small, hyalinized germinal centers and broad expansion of the mantle zone. Large, pleomorphic follicular dendritic cells are seen. In the plasma cell type, the interfollicular areas contain sheets of mature plasma cells. Although these findings are characteristic, they are not specific, and ultimately CD is a diagnosis of exclusion.

There are few descriptions of the cytomorphology of CD in FNA specimens, but some investigators suggest that dysplastic follicular dendritic cells may be a clue to the diagnosis.^{48,49} The presence of tissue fragments containing branching capillaries or hyaline material has been proposed as a cytologic hallmark of CD, but similar vascular branching can be seen in reactive lymphoid hyperplasia. Many believe that a specific diagnosis by FNA is not possible even with immunophenotypic analysis.⁴⁸

Lymphadenopathy caused by *Toxoplasma gondii* is most commonly cervical and usually self-limited, but it can mimic lymphoma clinically. The histologic changes are highly suggestive: follicular hyperplasia with small aggregates of epithelioid histiocytes that hug the periphery of germinal centers and zones of monocytoid B-cell hyperplasia. These architectural features are largely unappreciated in smears, which resemble reactive hyperplasia. The diagnosis is usually confirmed by serologic titers. It is only when organisms are seen—a rare occurrence—that a confident cytologic diagnosis can be issued.^{50,51} The organisms occur as bradyzoites within enlarged finely granular histiocytes or as free tachyzoites lying within an exudate, and their presence can be confirmed by immunocytochemistry⁵¹ or PCR.

FNA is extremely helpful in the identification of infectious and neoplastic causes of lymphadenopathy in patients with acquired immune deficiency syndrome (AIDS).^{52,53} An FNA diagnosis of HIV-associated lymphadenopathy (HIVAL), however, is one of exclusion. HIVAL is a primary, persistent nodal enlargement that is not a result of an opportunistic infection or neoplasm. It begins with follicular hyperplasia, followed by

regressive changes. The cytologic picture mimics reactive lymphoid hyperplasia.⁵⁴ Cytologically, one is often unable to document the explosive follicular hyperplasia with follicle lysis of the early phase, the follicular involution, partial lymphocyte depletion, and Castleman-like changes of the mid phase, or the small depleted follicles and paracortical vascular hyperplasia of late phase reactions.⁵⁵ Occasional multinucleated polykaryocytes are found in HIVAL smears, but they are non-specific.

Progressively transformed germinal centers (PTGC) is a benign condition marked by follicle expansion, disruption, or replacement by mantle cells, which are small-sized to intermediate-sized lymphocytes with round or irregular nuclei, inconspicuous nucleoli, and scant cytoplasm. PTGC is virtually impossible to identify as such by FNA. By FCM, one third of PTGC cases show a significant proportion of CD4+CD8+ (“double positive”) T-cells, which can be a clue to the diagnosis, but such populations can also be seen in T-cell lymphoma, thymoma, and nodular lymphocyte predominant Hodgkin lymphoma.⁵⁶ In fact, PTGC is often seen in patients with nodular lymphocyte predominant Hodgkin lymphoma, but most patients with PTGC do not develop lymphoma.

Dermatopathic lymphadenopathy is a nonspecific nodal enlargement associated with many chronic dermatoses, including psoriatic erythroderma, exfoliative dermatitides, and mycosis fungoides. The marked paracortical expansion by lymphocytes, dendritic cells, and histiocytes, seen in tissue sections as large pale nodules, is undetectable in smears. If melanin-laden macrophages are readily apparent, and few or no tingible-body macrophages are seen, the diagnosis can be strongly suggested.⁵⁷ Otherwise, most examples mirror reactive lymphoid hyperplasia. FNA, combined with PCR, may play a role in patients with mycosis fungoides in distinguishing dermatopathic lymphadenopathy from lymph node involvement by lymphoma.⁵⁸

Inflammatory and Infectious Conditions

Sarcoidosis

Sarcoidosis is a systemic granulomatous disease of unknown cause affecting young to middle-age adults and is more prevalent in women and African Americans. A wide variety of tissues may be involved, but the lung and lymph nodes of the mediastinum are most often affected. Peripheral lymphadenopathy is most common in the head and neck.⁵⁹ Noncaseating granulomas are a characteristic but nonspecific feature of sarcoidosis—they are also encountered in fungal, bacterial and, mycobacterial infections, hypersensitivity conditions, the drainage pathway of a malignancy, and exposures to foreign material—and the diagnosis is one of exclusion.

Calcium oxalate crystals and asteroid bodies are rarely seen in aspirates.⁶⁰ Aspirates from even large nodes may be sparsely cellular in the late phase of the disease (as a result of fibrosis).

CYTOMORPHOLOGY OF SARCOIDOSIS:

- granulomas
- epithelioid histiocytes
- multinucleated giant cells
- lymphocytes
- hypocellular aspirate (late phase)
- clean background

The key component of a granuloma is the epithelioid histiocyte, with its elongated, elliptical or spindle-shaped, curved or slightly indented nucleus. This produces a nuclear outline described as a “footprint,” “C-shape,” “V-shape,” or “boomerang shape” (Fig. 11.5). The number of granulomas is variable, and the number of epithelioid histiocytes in a granuloma varies from 5 to 50 or more.

DIFFERENTIAL DIAGNOSIS OF SARCOIDOSIS:

- infections
- foreign-body reaction
- spindle cell neoplasm
- granulomas accompanying a malignant neoplasm
- dendritic-lymphocytic aggregates

Infectious organisms must be excluded by special stains or microbiologic culture results. Most fungal and mycobacterial infections are not purely granulomatous, but they are accompanied by necrotic debris and a neutrophilic, lymphocytic, or plasmacytic infiltrate. The cells of spindle cell sarcoma, although often in aggregates, have atypical nuclei with coarsely textured chromatin, and footprint-shaped nuclei are uncommon in spindle cell tumors. The dendritic cells in dendritic-lymphocytic aggregates have round to oval nuclei rather than elongated, curved nuclei.

Granulomas accompany some malignancies (e.g., squamous cell carcinoma, Hodgkin lymphoma, and seminoma) more often than others (adenocarcinoma, sarcoma). This is generally not problematic because the malignant cells are clearly seen and distinct from the granulomas. Infrequently, however, the granulomatous response is so pronounced it obscures malignant cells, or the malignant cells resemble epithelioid histiocytes.⁶¹ A diagnosis of atypical or suspicious is justified in such borderline cases.

Bacterial and Fungal Lymphadenitis

Aspirates of acute bacterial lymphadenitis are hypercellular and composed of nearly a pure population of

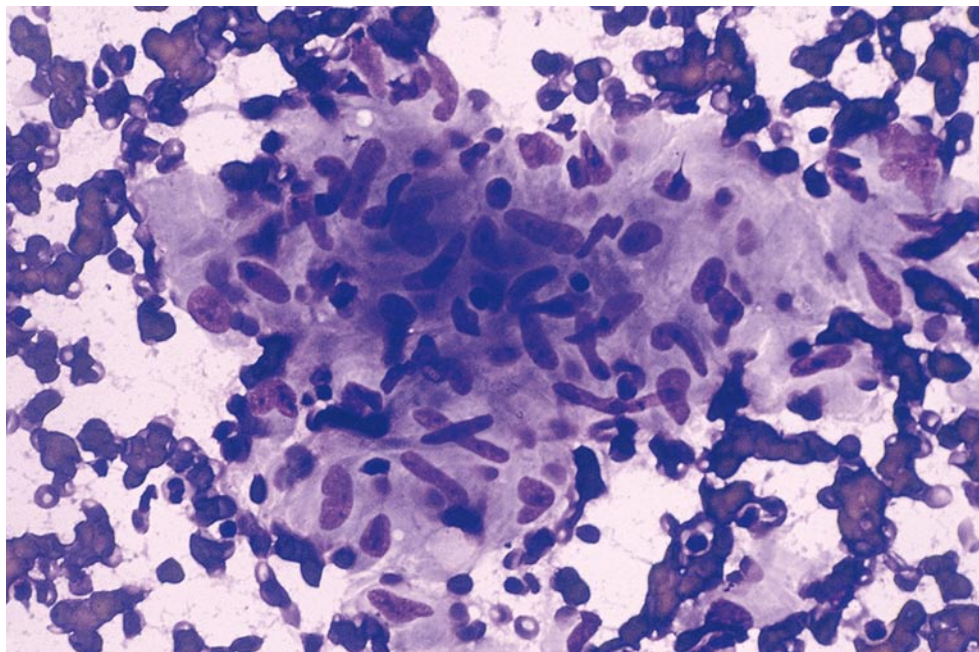


Figure 11.5 Sarcoidosis. In sarcoidosis, the granulomas are tight aggregates of epithelioid histiocytes. The cells have oval or curved nuclei and abundant cytoplasm with indistinct borders (Romanowsky stain).

neutrophils. Pus is often seen in the hub or barrel of the syringe at the time of the procedure. One should submit a part of any purulent biopsy for culture, expelling it into a sterile container or Culturette device. This should be done even if a patient is receiving antibiotics, because the organisms may be resistant to the patient's current antibiotic therapy. In some examples, the organisms are seen directly on the Romanowsky-stained smear (Fig. 11.6).

Aspirates of fungal lymphadenitis are variable in morphology. Some have only a pure neutrophilic infiltrate, some only granulomas, some a mixture of the two, and in some only the fungal organisms are present with few if any accompanying inflammatory cells. Among the more common fungi are *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Coccidioides immitis*.

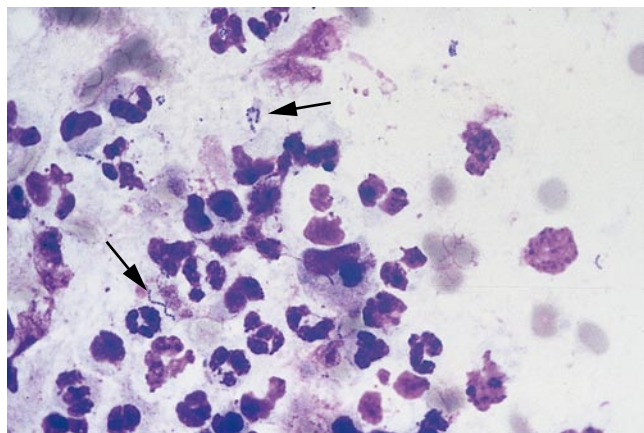


Figure 11.6 Acute lymphadenitis. Neutrophils are mixed with small round lymphocytes. Note the chains of bacterial cocci at the left and top of the image (Romanowsky stain).

Definitive diagnosis depends on histochemical stains (methenamine silver, acid fast, periodic acid-Schiff [PAS]), which can be performed directly on destained or unstained smears or cell block sections.

Cat Scratch Disease

Cat scratch disease is one of the most common causes of chronic, benign lymphadenopathy in North America. It is self-limited (resolving over a period of months in most patients) and primarily affects lymph nodes in the axilla, inguinal region, and neck of children and adolescents. Nodes are typically tender and may be matted together. The clinical history reveals a recent cat bite or scratch (often from a newly acquired kitten) in about 50% to 75% of cases. Early lesions begin with a proliferation of plasmacytoid monocytes, with small foci of necrosis and neutrophils. The necrotic foci enlarge and coalesce to form necrotizing granulomas called stellate microabscesses.

CYTOMORPHOLOGY OF CAT SCRATCH DISEASE:

- neutrophils
- loose or tightly clustered granulomas
- necrosis

Smears show numerous neutrophils and variably sized granulomas^{62,98} (Fig. 11.7). If only small granulomas are present, they may be obscured by the abundant neutrophils. The causative agent, the gram-negative bacillus *Bartonella henselae*, is stained using the Steiner and Steiner method, but results are highly variable.⁶⁴ Unless

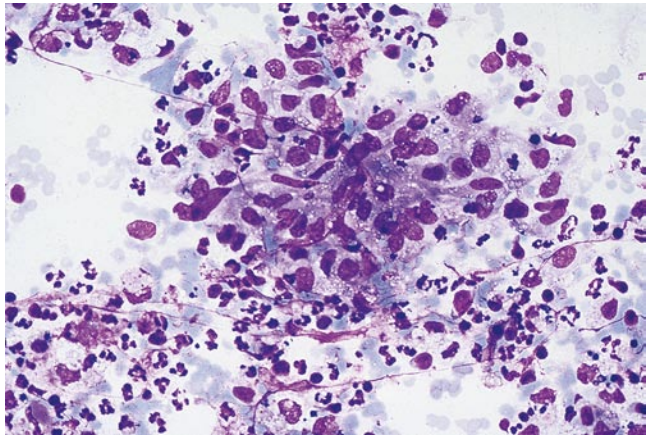


Figure 11.7 Cat scratch disease. A discrete aggregate of epithelioid histiocytes is surrounded by large numbers of neutrophils (Romanowsky stain).

the organism is identified or isolated, the cytologic diagnosis is presumptive, not definitive. Serologic studies can be helpful.

DIFFERENTIAL DIAGNOSIS OF CAT SCRATCH DISEASE:

- other causes of suppurative granulomatous lymphadenitis
- reactive lymphoid hyperplasia

Other organisms can produce an identical cytologic picture including *Francisella tularensis*, which causes tularemia, *Chlamydia trachomatis*, which causes lymphogranuloma venereum, *Yersinia enterocolitica* or *Y. pseudotuberculosis*, certain fungi, and less commonly *Mycobacterium tuberculosis*. In most cases, microbiologic or serologic analysis is required for specific diagnosis. Early stages of cat scratch disease

display few granulomas and mimic reactive lymphoid hyperplasia.⁶³

Mycobacterial Lymphadenitis

Mycobacterial infections occur in individuals who are immunocompetent and immunosuppressed. FNA is particularly efficacious in those countries where mycobacterial infection is endemic; its accuracy may be lower in the United States because of the lower prevalence of disease and atypical clinical presentations.⁶⁵

CYTOMORPHOLOGY OF MYCOBACTERIAL LYMPHADENITIS:

- necrosis
- granulomas
- histiocytes
- neutrophils
- intracellular and extracellular bacilli (“negative images”)
- acid-fast staining of bacilli

Smears may show granulomas with necrosis, granulomas without necrosis, or sometimes necrosis only⁶⁶ (Fig. 11.8A). In patients who are immunocompromised, there may be only loose aggregates of histiocytes rather than true granulomas. The “negative image” phenomenon⁶⁷ occurs because the lipid coat of the bacillus resists staining with any Romanowsky stain; the bacilli are seen as optically clear rods or striations surrounded by stained proteinaceous or necrotic material. These rods may be extracellular or within macrophages, where they appear as multiple linear striations resembling the “crinkled tissue paper” appearance of the storage cells of Gaucher disease. This phenomenon is not visible with the Papanicolaou stain.

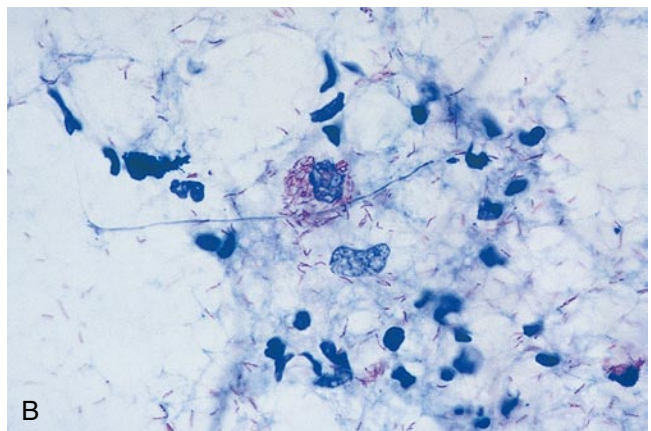
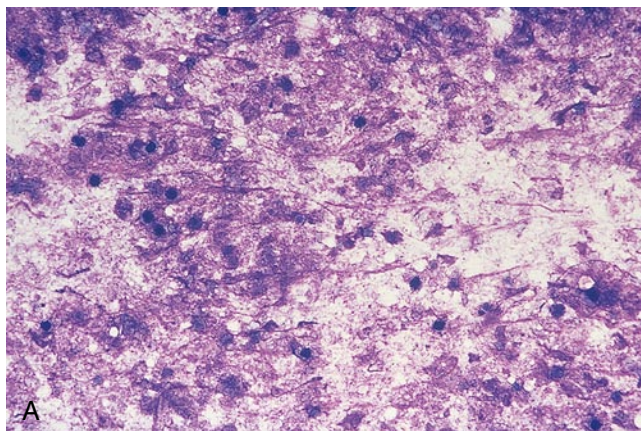


Figure 11.8 Tuberculous lymphadenitis. A, Necrotic material and only few degenerating nuclei (Romanowsky stain). B, A stain for acid-fast bacilli shows many extracellular bacilli and a macrophage filled with organisms (Ziehl-Neelsen stain.)

DIFFERENTIAL DIAGNOSIS OF MYCOBACTERIAL LYMPHADENITIS:

- sarcoidosis
- granulomatous lymphadenitis resulting from other organisms
- lymph node infarction

Cases of mycobacterial lymphadenitis with little or no necrosis resemble sarcoidosis. Special stains for bacteria, acid-fast bacilli, and fungi are important whenever granulomas, necrosis, or a neutrophilic infiltrate is present. Importantly, organisms are best seen with special stains in necrotic material (Fig. 11.8B), but the sensitivity of acid-fast bacilli stains is low. Molecular techniques can detect and speciate mycobacteria. Alternatively, if necrosis or granulomas are seen at the time of the rapid evaluation of specimen adequacy, a portion of the needle rinse can be submitted for microbiologic cultures.

Complete or subtotal lymph node infarction is uncommon, but occurs in malignant lymphoma, systemic lupus erythematosus (SLE), vascular thrombosis, trauma, and infection. In such cases, smears may contain amorphous proteinaceous material with or without inflammatory cells, and the outlines of cell “ghosts” may be seen, but stains for organisms are negative.

Rosai-Dorfman Disease (Sinus Histiocytosis with Massive Lymphadenopathy)

Rosai-Dorfman disease (RDD) is an uncommon nodal and extranodal disease primarily of young children and adolescents, although a wide age spectrum is affected. The classic clinical presentation is bilateral, painless cervical lymphadenopathy. Accompanying symptoms include fever, joint pain, night sweats, and even weight loss—all of which can mimic lymphoma clinically. Laboratory findings include polyclonal hypergammaglobulinemia and leukocytosis. Histologic sections show marked distension of sinuses by large histiocytes.

CYTOMORPHOLOGY OF ROSAI-DORFMAN DISEASE:

- small lymphocytes
- histiocytes with emperipolesis

Small lymphocytes vastly outnumber transformed lymphocytes, plasmacytoid lymphocytes, and immunoblasts. The histiocytes are large, with large pale nuclei, nucleoli, and abundant vacuolated cytoplasm; they can simulate the cells of an epithelial neoplasm. The key cytomorphic feature is “emperipolesis,” the engulfment of lymphocytes by histiocytes (Fig. 11.9), but it may

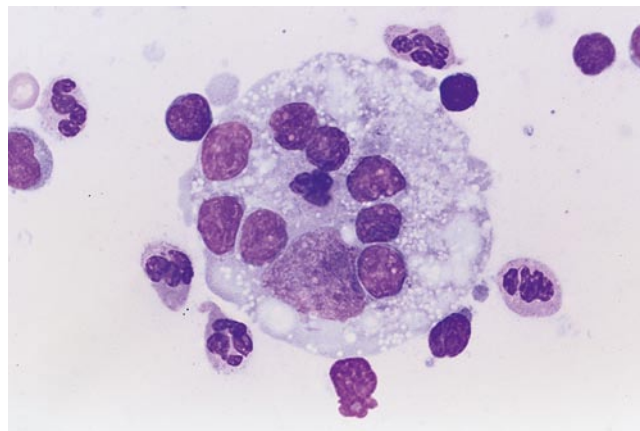


Figure 11.9 Rosai-Dorfman disease (RDD). An enormous histiocyte with two overlapping enlarged nuclei has engulfed many small lymphocytes (Romanowsky stain). (Courtesy of Dr. Harry Kozakewich, Children's Hospital Medical Center, Boston.)

be difficult to appreciate because engulfed lymphocytes can be so numerous that they obscure the underlying histiocyte nucleus.^{68,69} The large histiocytes are immunoreactive for S-100 protein and CD68.

DIFFERENTIAL DIAGNOSIS OF ROSAI-DORFMAN DISEASE:

- reactive lymphoid hyperplasia
- Langerhans cell histiocytosis

Dendritic-lymphocytic aggregates in reactive lymphoid hyperplasia mimic histiocytes with emperipolesis. Instead of being engulfed by histiocytes, however, lymphocytes overlie and commingle with them in reactive hyperplasia. Because many of the lymphocytes “spill” from the cytoplasm as a consequence of smearing in RDD, they are often not strictly within the cytoplasm, but adjacent to it, thus adding to the confusion. An additional helpful feature is that histiocytes are much more numerous in RDD than in a reactive lymph node. Also, the histiocytes in dendritic-lymphocytic aggregates are negative for S-100 protein.

Langerhans cell histiocytosis, primarily a disorder of children, uncommonly involves lymph nodes without simultaneously or previously also involving bone, lung, or another site. The neoplastic cells, like the cells of RDD, are positive for S-100 protein but, unlike RDD, are also positive for CD1a. Cytologic features are helpful in this distinction: unlike the round nuclei of RDD histiocytes, those of Langerhans cell histiocytosis have reniform and contorted shapes, lack nucleoli, and display partial or complete linear grooves. There is no emperipolesis. Smears of Langerhans cell histiocytosis may also contain increased numbers of eosinophils.

Kikuchi Lymphadenitis

Kikuchi lymphadenitis (KL), also known as histiocytic necrotizing lymphadenitis, is a self-limited adenopathy of unknown cause that occurs primarily in patients who are Asian. Patients are usually young and often present with cervical adenopathy, fever, lymph node tenderness, and an atypical peripheral lymphocytosis. Laboratory test results are non-specific. Clinically, the patient with KL is often suspected of having an acute bacterial lymphadenitis or abscess and is treated empirically with antibiotics. The cytologic diagnosis of KL can be reassuring because the disease is self-limited, and antibiotics are unnecessary.

CYTOMORPHOLOGY OF KIKUCHI LYMPHADENITIS:

- necrotic debris, karyorrhexis
- small phagocytic histiocytes with sharply angulated (crescent-shaped) nuclei
- cytoplasmic tingible bodies
- an increased number of immunoblasts and plasmacytoid monocytes
- absence of neutrophils

Smears from patients with KL have a characteristic appearance that permits diagnosis by FNA in the proper clinical context.⁷⁰ Granular proteinaceous and nuclear debris is admixed with distinctive histiocytes with a contorted, sharply angulated, often crescent-shaped, peripherally placed nucleus^{70,71} (Fig. 11.10). These histiocytes are readily distinguished from tingible-body macrophages, which are larger and have round nuclei. Plasmacytoid monocytes—medium-sized cells with an

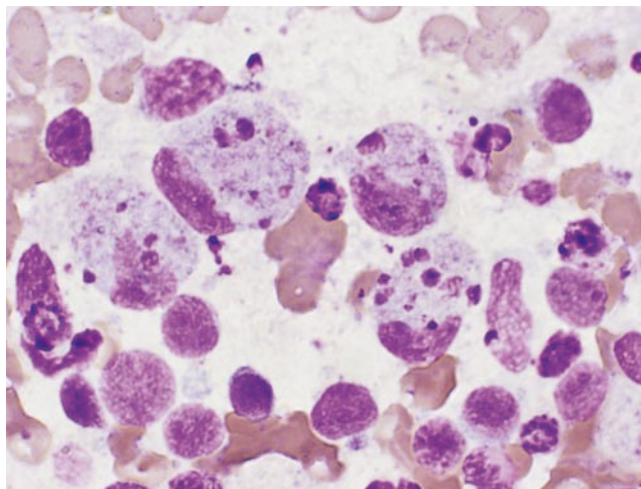


Figure 11.10 Kikuchi lymphadenitis. Histiocytes with contorted nuclei, including one with a “C-shape,” contain ingested debris. These cells are significantly smaller than tingible-body macrophages (Romanowsky stain).

eccentrically placed round nucleus, condensed chromatin, and a moderate amount of cytoplasm—are also present, as are immunoblasts. Neutrophils are sparse or absent. No lymphocytic emperipolesis is present.

DIFFERENTIAL DIAGNOSIS OF KIKUCHI LYMPHADENITIS:

- reactive follicular hyperplasia (with tingible body macrophages)
- tuberculous lymphadenitis
- necrotizing (suppurative) granulomatous lymphadenitis
- SLE

The combination of necrosis and contorted small phagocytic histiocytes, in the absence of neutrophils, is quite striking. The characteristic small, contorted phagocytic histiocytes are easily overlooked on casual examination, but they are highly characteristic of KL and only rarely occur in other conditions.⁷⁰ If misinterpreted as tingible-body macrophages, an erroneous diagnosis of reactive follicular hyperplasia might be rendered. In the proper clinical context, the FNA report can strongly suggest the diagnosis of KL because the small phagocytic histiocytes are morphologically distinguishable from the larger tingible-body macrophages. Similar small phagocytic histiocytes can be seen in tuberculous lymphadenitis,⁷⁰ a diagnosis that should be excluded with the help of histochemical stains and microbiologic cultures. A variety of bacterial and fungal infections can result in a necrotizing, suppurative, and granulomatous lymphadenitis. A suppurative lymphadenitis contains abundant neutrophils that are not seen in KL. The epithelioid histiocytes of a granulomatous lymphadenitis have elliptical, indented nuclei with smooth, rounded contours that are distinct from the angulated edges of the histiocytic nuclei of KL. Lupus lymphadenitis is morphologically similar to KL.⁷² Smears from lupus lymphadenitis typically contain “hematoxylin bodies”—darkly stained clumps of nuclear debris measuring 1 to 100 μ m in diameter that have not been described in patients with KL.⁷² Even if hematoxylin bodies are absent from smears, it is prudent to exclude lupus with serologic testing whenever the FNA findings suggest KL.

Infectious Mononucleosis

Infectious mononucleosis is caused by the EBV and spread through person-to-person contact, most commonly in adolescents and young adults. The clinical presentation may include fever, malaise, pharyngitis, rash, and peripheral lymphadenopathy, typically cervical, but axillary and inguinal lymphadenopathy also occur. Lymph nodes are often tender and movable. Splenomegaly is common, and splenic rupture is

a potential complication. Laboratory findings include peripheral blood atypical lymphocytosis, and a positive heterophil (Monospot) test.

CYTOMORPHOLOGY OF INFECTIOUS MONONUCLEOSIS:

- increased percentage of immunoblasts, plasmacytoid lymphocytes, and plasma cells
- few dendritic-lymphocytic aggregates and tingible-body macrophages

Aspirates contain predominantly small lymphocytes, accompanied by an increased number of immunoblasts, centroblasts, plasmacytoid lymphocytes, and occasionally plasma cells^{73,74} (Fig. 11.11). Binucleated immunoblasts simulating RS cells may be found but are rare. IP confirms the polyclonal nature of the B-cells, and often shows a reversed CD4/CD8 ratio.

DIFFERENTIAL DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS:

- reactive lymphoid hyperplasia
- other lymphadenopathy associated with increased immunoblasts
- large cell lymphoma
- Hodgkin lymphoma

A high percentage of immunoblasts, centroblasts, and plasmacytoid lymphocytes are typical of infectious mononucleosis, but the amount depends on the stage of the disease. In the early stages of infectious mononucleosis, immunoblast proliferation is barely perceptible, and FNA is indistinguishable from reactive lymphoid

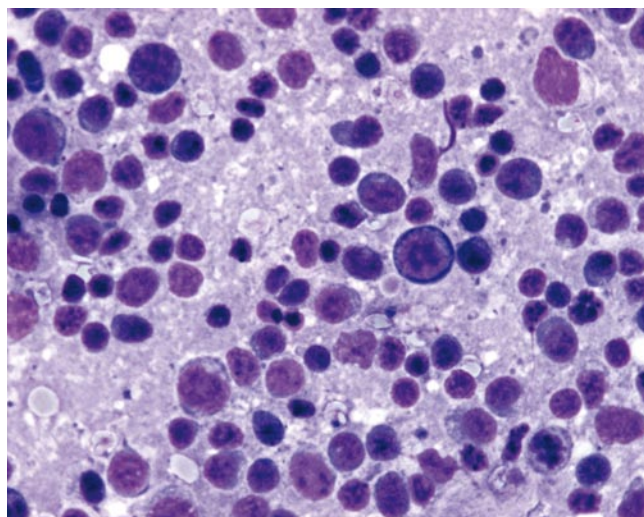


Figure 11.11 Infectious mononucleosis. Many immunoblasts are mixed with small round lymphocytes and plasmacytoid lymphocytes (Romanowsky stain).

hyperplasia.⁷⁴ The immunoblast proliferation in other lymphadenopathies (e.g., anticonvulsant-associated lymphadenopathy, herpes simplex, cytomegalovirus, and postvaccinal lymphadenitis) and drug hypersensitivity is indistinguishable from that of infectious mononucleosis. A detailed clinical history, serologic studies, or excisional biopsy may be necessary to exclude these other entities. Most patients who develop anticonvulsant-related lymphadenopathy (principally to phenytoin) usually do so within 6 months of the onset of therapy. Herpetic lymphadenitis usually occurs in the setting of disseminated infection in a patient who is immunosuppressed, and diagnosis is usually made by association with the cutaneous lesions. In addition to increased immunoblasts, herpes simplex and cytomegalovirus lymphadenitis may contain cells with diagnostic viral inclusions.

Because infectious mononucleosis induces a discernible increase in large cells and there is a scarcity of tingible-body macrophages and dendritic-lymphocytic aggregates, one of the “large cell” NHLs needs to be considered. Immunophenotypic studies are helpful in problematic cases to confirm the polyclonal nature of the immunoblasts in infectious mononucleosis. Immunoblasts are occasionally mistaken for mononuclear RS cell variants. Unlike RS cells, immunoblasts are smaller, have smaller nucleoli, greater cytoplasmic basophilia, and sometimes contain a perinuclear zone of clearing. Binucleated immunoblasts mimicking classic RS cells are extremely rare.

NEOPLASMS

Lymph node FNA is well accepted for the diagnosis of metastatic tumors and recurrent lymphoma and is achieving acceptance for the primary diagnosis of malignant lymphoma. Although different viewpoints exist,^{15,75} there is a broad consensus among cytopathologists that FNA is a valuable tool in the primary diagnosis and classification of lymphoma,^{18,20,27,28,76} but only if used by individuals that apply IP, develop a thorough understanding of the WHO classification, and understand the limitations of the technique.

Fine-needle aspiration is a valuable tool in the primary diagnosis and classification of lymphoma if the cytopathologist:

- understands modern lymphoma classification
- uses special studies (IP, molecular genetics)
- works closely with a hematopathologist

According to the WHO classification of hematologic malignancies, lymphoid neoplasms are defined by a combination of morphology, immunophenotype,

genetic features, and clinical findings.¹⁶ There is no single gold standard for all lymphomas; instead, for each disease, one or more of these may be more important for diagnosis than the others. Architectural pattern (follicular versus diffuse), important in previous classification systems, is no longer of paramount importance. This plays into the strengths of FNA, and the new classification has been embraced by cytopathologists.⁷⁷

A primary diagnosis of lymphoma almost always requires precise classification. Unqualified diagnoses such as lymphoma, small cell lymphoma, or large cell lymphoma are usually not refined enough for patient management, and given such nonspecific diagnoses, an oncologist will likely demand an open biopsy. Thus, in contrast to a diagnosis like metastatic squamous carcinoma, typically accomplished by light microscopic examination of routinely stained cytologic preparations only, lymphoma diagnosis by FNA requires more time and effort, special handling, and the use of ancillary markers applied in a systematic fashion.⁷⁸

A comprehensive lymph node fine-needle aspiration work-up includes:

- clinical history
- rapid evaluation
- good smears
- needle rinse for:
 - FCM or immunocytochemistry
 - molecular genetic studies (selected cases)
 - tissue fragments for architecture, immunohistochemistry (selected cases)

Hodgkin Lymphoma

Hodgkin lymphoma (HL), also called Hodgkin disease, is a common cause of malignant lymphadenopathy, representing approximately 30% of all lymphomas. Table 11.3 shows the current WHO classification of HL. It comprises two distinct diseases: classical HL and nodular lymphocyte predominant HL. They are bound by a shared characteristic; within a lesion, the neoplastic cells are outnumbered by non-neoplastic inflammatory cells. They differ, however, in their clinical features, immunophenotype, genetics, and natural history.

TABLE 11.3 WORLD HEALTH ORGANIZATION'S CLASSIFICATION OF HODGKIN LYMPHOMA

Classical Hodgkin Lymphoma

- Nodular sclerosis classical Hodgkin lymphoma
- Lymphocyte-rich classical Hodgkin lymphoma
- Mixed cellularity classical Hodgkin lymphoma
- Lymphocyte-depleted classical Hodgkin lymphoma

Nodular Lymphocyte Predominant Hodgkin Lymphoma

The *classical subtype* accounts for 95% of HL. Epidemiologic studies show a bimodal age curve with a peak at 15 to 35 years of age and a smaller peak later in life. The malignant cells—large multinucleated RS cells and their mononuclear variants—reside in an infiltrate of non-neoplastic small lymphocytes, eosinophils, neutrophils, histiocytes, plasma cells, and fibroblasts. The proportions of the various non-neoplastic elements vary. In virtually all cases, the RS cells are monoclonal B cells as demonstrated by IP and molecular genetics. RS cells are positive for CD30 in almost all cases and for CD15 in 75% to 85%. CD20 is detected in about 40% and often expressed in only a minority of the RS cells. Epithelial membrane antigen (EMA) positivity is seen in less than 5% of cases, and CD45 is usually negative in the RS cells.

Four histologic variants of classical HL have been recognized (see Table 11.3). Distinction among these variants has no clinical relevance, and thus it is no great loss that they cannot be separated by FNA. In the past, most oncologists required that a newly diagnosed patient have an excisional biopsy for confirmation of the FNA diagnosis. Today an FNA diagnosis of classical HL that is confirmed with immunostains requires no further tissue confirmation or subclassification for patient management.⁷⁹

Nodular lymphocyte predominant HL (NLPHL) is much less common than classical HL, with different clinical features and natural history. Most patients are male and between 30 and 50 years of age. The malignant cells are monoclonal B cells called L & H cells: large cells with one large, often folded or multilobate nucleus. The nuclei of these cells are so contorted they are commonly called popcorn cells. The nucleoli are usually smaller than those in classical RS cells. L & H cells are positive for CD20 and CD45 in almost all cases and for EMA in up to 50%. They are almost always negative for CD15 and CD30, unlike the RS cells in classical HL. The architecture is partially or totally nodular. This architectural feature is important in the distinction from a T-cell-rich large B-cell lymphoma (TCRLBL) and thus limits the ability of FNA to make this distinction. The FNA features of NLPHL are not well documented because the disease is so uncommon.

CYTOMORPHOLOGY OF HODGKIN LYMPHOMA:

- small lymphocytes
- eosinophils (especially in the mixed cellularity subtype)
- Reed-Sternberg cells, classic and mononuclear variants (classical HL)
- L & H cells (NLPHL)
- no lymphohistiocytic aggregates or tingible-body macrophages (exceptions partial node involvement and lymphocyte predominant HL)

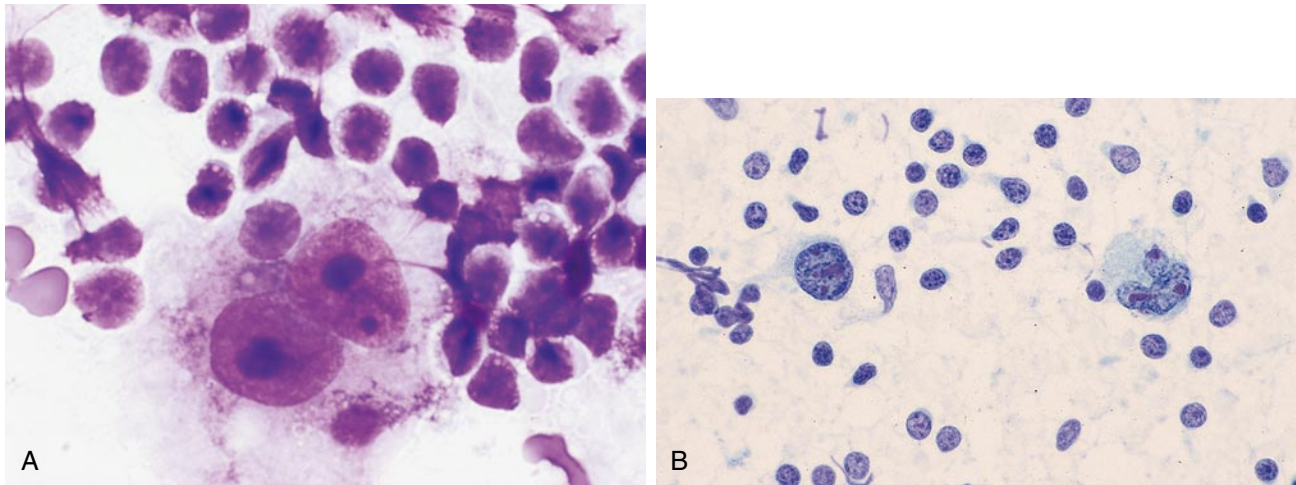


Figure 11.12 Classical Hodgkin lymphoma (HL). A, A classic binucleated Reed-Sternberg (RS) cell (Romanowsky stain). B, Two conspicuous mononuclear RS cells are surrounded by lymphocytes. The RS nucleus at the right is lobulated, while the one to the left has only slight bosselation. Both have macronucleoli (Papanicolaou stain).

The diagnosis of classical HL is established by finding the rare RS cells in a mixed background of small lymphocytes and eosinophils (Fig. 11.12A). Thus, FNA samples with conspicuous eosinophils should be carefully screened for RS cells. Diagnostic, binucleated and multinucleated RS cells have at least two nuclei or nuclear lobes, each with an enormous nucleolus. Binucleated and multinucleated RS cells are infrequent in many cases. A clue to the diagnosis of classical HL in such cases is the more frequently encountered mononuclear RS cell variant (Fig. 11.12B). The mononuclear variant is 3 to 4 times the size of a small lymphocyte and has a round, irregular, or polylobated nucleus.¹³ Large, bare nuclei of the mononuclear RS cell variants are sometimes prominent at medium power; their presence is helpful in raising the possibility of classical HL. Sometimes, however, they are masked in a densely lymphocytic smear. The problem is exacerbated when the node is only partially involved by classical HL. Granulomas are sometimes present, but are typically small, inconspicuous, and poorly formed.

The cytologic findings in NLPHL are not as well documented. In many ways, NLPHL resembles classical HL in that the neoplastic L & H (popcorn) cells are outnumbered by a non-neoplastic, reactive population of small B and T lymphocytes and follicular dendritic cells. L & H cells are large cells with a single, large, highly folded or multilobate nucleus that often look like a popcorn (Fig. 11.13). Nucleoli are not as prominent as in RS cells. It is fair to say that the diagnosis of NLPHL is extremely challenging to make by FNA. An unequivocal diagnosis, in fact, is rarely made. Some cases are called atypical or suspicious, but false-negative interpretations do occur.⁵⁶ By FCM, NLPHL often has a significant proportion of CD4+CD8+ (double positive) T-cells,

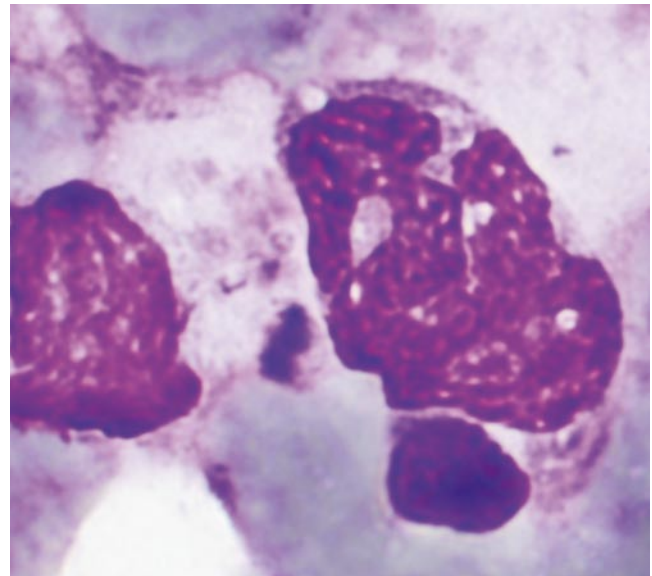


Figure 11.13 Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). The characteristic cell is the lymphocytic and histiocytic (L & H) i.e., popcorn cell, which has a large, folded nucleus. One must hunt for these cells because they are vastly outnumbered by small lymphocytes and other non-neoplastic lymphoid cells (Romanowsky stain).

which is rare in most reactive lymphadenopathies and can be a clue to the diagnosis,⁵⁶ but a double-positive T-cell population is also sometimes seen in PTGC and T-cell lymphomas. Knowing that such double-positive T-cells are rare in reactive lymph nodes (except in PTGC), finding a double-positive population by FCM should prompt consideration of excisional biopsy for definitive diagnosis.

DIFFERENTIAL DIAGNOSIS OF HODGKIN LYMPHOMA:

- reactive lymphoid hyperplasia
- infectious mononucleosis
- T-cell-rich large B-cell lymphoma
- anaplastic large cell lymphoma
- acute lymphadenitis (rare)
- nasopharyngeal carcinoma

A major pitfall is a hypocellular smear. Because nodular sclerosis is the most common classical HL subtype, an inability to aspirate sufficient numbers of diagnostic RS cells is a source of false-negative interpretations.⁸⁰ There may be few RS (or L & H) cells in smears that otherwise resemble *reactive lymphoid hyperplasia* (e.g., lymphocyte-rich HL, NLPHL). A search for RS cells and L & H cells is warranted whenever a lymph node has the features of reactive lymphoid hyperplasia or conspicuous eosinophils, particularly if the node is clinically suspicious.

Mononuclear RS cells resemble immunoblasts, and therefore benign lesions with conspicuous immunoblasts, like *infectious mononucleosis*, must be considered in the differential diagnosis. Immunoblasts are smaller than RS cells and often can be distinguished simply on the basis of their size, but clinical correlation, microbiologic/serologic testing, and special stains can be helpful in difficult cases. The CD4/CD8 ratio by FCM can also be helpful. In contrast with reactive or infectious lymphadenopathies like IM, in which the ratio shifts in favor of CD8+ T-cells, in HL there is an excess of CD4+ T-cells.⁸¹ A CD4/CD9 ratio ≥ 5 , although not by itself diagnostic of HL, should alert the cytologist to search carefully for morphologic evidence of HL.⁸¹

T-cell-rich large B-cell lymphoma (TCRLBL) mimics HL in that the neoplastic cells are outnumbered by abundant non-neoplastic small lymphocytes. Immunostains are helpful because the malignant cells of TCRLBL are usually uniformly positive for CD20 and negative for CD30 and CD15, making classical HL highly unlikely. The distinction between TCRLBL and NLPHL is difficult if not impossible by FNA and may require nodal excision with assessment of nodular versus diffuse architecture. By FCM, NLPHL often has a significant proportion of CD4+CD8+ (double positive) T-cells, which can be a clue to the diagnosis.⁵⁶ A double-positive T-cell population is also sometimes seen in PTGC and T-cell lymphomas.

The neoplastic cells in *anaplastic large cell lymphoma* are sometimes indistinguishable from RS and L & H cells. In anaplastic large cell lymphoma, these cells may be much more numerous, whereas in HL, RS and L & H cells represent only 0.1% to 10% of the total cell population.⁸² In borderline cases, immunostains

for EMA and ALK protein (positive in anaplastic large cell lymphoma, negative in classical HL) and, if necessary, gene rearrangement studies for T-cell receptors are helpful.

Classical HL associated with suppuration can mimic *acute lymphadenitis* because of the abundance of neutrophils and cell debris.⁸³ A painstaking search reveals large RS cell variants scattered among the acute inflammatory cells.

Metastatic nasopharyngeal carcinoma has an abundant lymphoid background and shows a predilection for cervical lymph nodes, with neoplastic cells that resemble RS cells; immunostaining for keratins is helpful in this distinction.

Non-Hodgkin Lymphoma

The WHO classification divides NHL into B- and T-cell types. In the United States, the great majority (90%) are B-cell neoplasms (Table 11.4). Two B-cell neoplasms—DLBL and follicular lymphoma—account for up to two thirds of all B-cell neoplasms. About 10% of NHLs are T-cell neoplasms, and a small minority is of null cell type. NHLs are also segregated by morphology and can be loosely subdivided into those composed of small cells versus large cells. Morphology is integrated with immunophenotypic results and clinical findings for a final subclassification.

TABLE 11.4 WORLD HEALTH ORGANIZATION'S CLASSIFICATION OF B-CELL NEOPLASMS*

Precursor B-Cell Lymphoblastic Leukemia or Lymphoma

Mature B-cell neoplasms

B-cell chronic lymphocytic leukemia or small lymphocytic lymphoma

B-cell prolymphocytic leukemia
Lymphoplasmacytic lymphoma

Mantle cell lymphoma

Follicular lymphoma

Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type

Nodal marginal zone B-cell lymphoma with or without monocytoid B-cells
Splenic marginal zone B-cell lymphoma
Hairy cell leukemia

Diffuse large B-cell lymphoma

Subtypes: mediastinal (thymic), intravascular, primary effusion

Burkitt lymphoma

Plasmacytoma

Plasma cell myeloma

*More common entities are in bold.

In discussing FNA diagnosis of NHL, it is helpful to review the small cell lymphomas separately from the large cell lymphomas. The differential diagnosis for each will be discussed at the end of each section.

TABLE 11.5 CLINICAL FEATURES OF SMALL B-CELL LYMPHOMAS

Type	Median age/gender	Stage I	Extranodal*	Clinical Course
Small lymphocytic	65/m > f	None	Rare	Indolent, incurable
Mantle cell	60/m > f	Rare	Many	Aggressive, incurable
Follicular	59/m = f	Rare	Variable	Indolent, rarely curable
Marginal zone/MALT	61/m = f	Often	Usually	Indolent, curable
Lymphoplasmacytic	63/m = f	Rare	Rare	Indolent, incurable

*Excluding bone marrow.

MALT, mucosa-associated lymphoid tissue.

Lymphomas of Small Cells

The clinical features of the five major NHLs comprised of small lymphoid cells are summarized in Table 11.5.

Small Lymphocytic Lymphoma. SLL comprises about 6% of NHL. It occurs in older adults and is indolent and incurable. Disease is widespread at the time of diagnosis, usually with peripheral blood and bone marrow involvement. SLL is morphologically and immunophenotypically identical to chronic lymphocytic leukemia (CLL), which often secondarily involves lymph nodes. Tissue sections show effacement of nodal architecture by the neoplastic cells with pseudofollicular growth centers that contain prolymphocytes and paraimmunoblasts. The CD5+, CD10–, CD23+ immunophenotype is highly characteristic of SLL and CLL.

CYTOMORPHOLOGY OF SMALL LYMPHOCYTIC LYMPHOMA:

- monomorphous small lymphocytes
- clumped (soccer-ball-like) chromatin
- smooth or minimally irregular nuclear contour
- inconspicuous or absent nucleoli
- scant cytoplasm
- prolymphocytes and paraimmunoblasts
- rare or absent tingible-body macrophages and dendritic-lymphocytic aggregates

Nuclei are round and have coarse, clumped chromatin—so-called clotted chromatin. Nuclear borders are smooth and usually round. Prolymphocytes are larger, have a distinct nucleolus, and have a modest amount of pale cytoplasm, whereas paraimmunoblasts are larger still, with a more prominent nucleolus, and more basophilic cytoplasm (Fig. 11.14). These two types of transformed lymphocytes are not easily distinguished from each other in smears.

Morphologic transformation to a large cell lymphoma occurs in up to 20% of patients with SLL and CLL and is an important indicator of prognosis. Transformation is assessed by determining the proportion of large cells

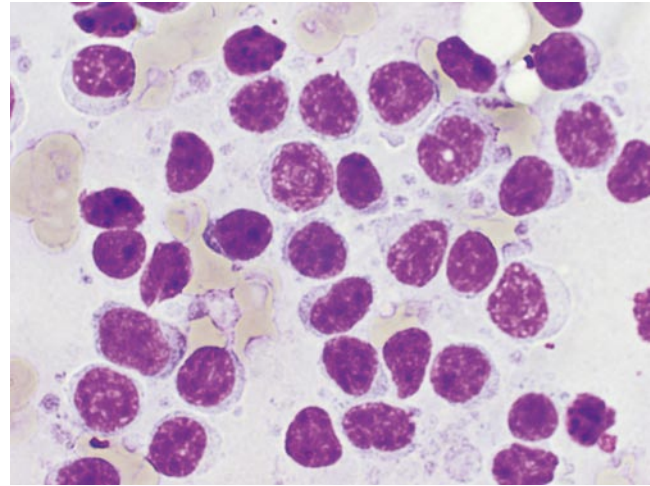


Figure 11.14 Small lymphocytic lymphoma (SLL). The monotonous neoplastic small lymphocytes have scant cytoplasm; some bare nuclei are present. The mostly round nuclei have dense (“clotted”) chromatin. Lymphoglandular bodies are present in the background (Romanowsky stain).

and identifying necrosis. Immunocytochemical staining with the Ki-67 proliferation marker can be a valuable adjunct to morphologic assessment of transformation. A Ki-67 index of >30% strongly suggests transformation.⁸⁴

Mantle Cell Lymphoma. Mantle cell lymphoma (MCL) is a biologically aggressive B-cell lymphoma comprising 6% to 8% of NHL. It occurs in adults over 50 years of age, is rare in children, and affects men much more often than women (5:1). MCL is commonly extranodal, and most patients have disseminated disease at initial diagnosis. MCL is resistant to standard lymphoma therapeutic regimens and has a poor prognosis, with a median survival of 2 to 5 years. A t(11;14)(q13;q32) translocation is present in the vast majority of patients, resulting in an up-regulation of the bcl-1 (PRAD1, CCND1) gene. This in turn leads to overexpression of the protein cyclin D1, which acts at the G₁ to S phase checkpoint of the cell cycle to drive cell proliferation. This translocation is readily detectable by FISH on cytologic material.^{85,86} The CD5+, CD10–, CD23– immunophenotype is characteristic of MCL.

CYTOMORPHOLOGY OF MANTLE CELL LYMPHOMA:

- monomorphous small cells
- fine nuclear chromatin
- irregular nuclear contours
- inconspicuous nucleoli
- scant cytoplasm
- no centroblasts or immunoblasts
- rare tingible-body macrophages
- lymphoid cell aggregates (one third of cases)
- blastoid variant

Aspirates of MCL are comprised of dispersed, uniform cells that have a high nuclear-to-cytoplasmic ratio and resemble centrocytes⁸⁷ (Fig. 11.15A). Lymphoid cell aggregates occur in about one third of cases.⁷⁶ They are similar to dendritic-lymphocytic aggregates, except that dendritic cells are inconspicuous.^{43,76} Unlike other small cell lymphomas, there are virtually no transformed lymphocytes. In the blastoid variant, the cells resemble lymphoblasts;⁸⁸ some studies suggest that it has a more aggressive course.¹⁶ The cytologic interpretation can be confirmed by demonstrating the characteristic CD5+, CD10–, CD23– immunoprofile by FCM; immunoreactivity for cyclin D1 on cytocentrifuge preparations; and/or the (11;14) translocation by FISH (Fig. 11.15B).

Follicular Lymphoma. Follicular lymphoma (FL) accounts for about 35% of adult NHL in the United States. Patients are generally older than 50 years, and the vast majority (>80%) have disseminated disease (spleen, lymph node, and bone marrow involvement) at the time of diagnosis. In 25% to 35% of patients with FL, transformation to a large B-cell lymphoma occurs. This usually portends rapid disease progression that is refractory to treatment.¹⁶

Histologically, FLs are divided into low-grade (1 and 2) and high-grade (3) tumors based on the quantity of large cells (centroblasts). The CD5–, CD10+ immunophenotype is characteristic of follicular lymphoma (CD23 can be + or –), but as many as 40% of FLs are negative for CD10²⁶ (Table 11.6). The tumor cells show nuclear immunoreactivity for bcl-6.

FL is characterized by a t(14;18)(q32;q21) translocation, with rearrangement of the bcl-2 gene, in up to 95% of cases. This translocation can be detected by FISH on cytologic material in over 80% of cases,⁸⁹ and FISH appears to be more sensitive than FCM in diagnosing FL.⁹⁰ Antibody staining with bcl-2, which is extremely helpful in distinguishing FL from follicular hyperplasia in tissue sections, is ineffective in smears because the spatial relationship of follicle center versus paracortical staining cannot be evaluated.

CYTOMORPHOLOGY OF FOLLICULAR LYMPHOMA:

- predominantly small irregular or cleaved lymphocytes
- large cleaved or noncleaved lymphocytes (more in high-grade follicular lymphoma)
- few tingible-body macrophages
- lymphoid cell aggregates (one third or more of cases)

Although some have claimed that a follicular pattern can be appreciated in well-made smears, our experience leads us to conclude that this is uncommon. Aspirates may be composed almost exclusively of small centrocytes, but they are more apt to show a mixture of centrocytes and some centroblasts (large noncleaved lymphocytes).⁷⁶ Cell nuclei are commonly contorted into notches and grooves. In some aspirates the clefts are so exaggerated as to appear to bisect or trisect the nucleus, whereas in others the nuclear folds are more subtle (Fig. 11.16). Dendritic-lymphocytic aggregates occur in FL in about one third of cases,⁷⁶ but the dendritic cells are sometimes difficult to discern.^{43,76} Several variants of FL have been described: FL with monocytoid B cells, FL with rosettes, floral pattern of FL, and FL with cerebriform nuclei. These variants have no clinical significance and generally are not recognizable by FNA. A signet ring cell variant of FL, however, is cytologically distinct because the cells have a single, large, optically clear cytoplasmic vacuole filled with immunoglobulin that can create confusion with a signet-ring-cell carcinoma.

There is no standardized method for grading a FL obtained by FNA. As a practical matter, this can be done by visual estimation of the percentage of small and large lymphocytes,⁹¹ by counting the number of centroblasts within aspirated follicles,⁹² or by counting centroblasts on liquid-based slides.⁹³ The distinction between grade 3 FL and DLBCL is usually not possible by FNA and may not be clinically relevant.

Marginal Zone Lymphoma. Marginal zone lymphoma (MZL) is an indolent, low-grade B-cell lymphoma that is divided into nodal and extranodal types. The extranodal type is termed mucosa-associated lymphoid tissue (MALT) lymphoma and is more common than the nodal type, accounting for 7% to 8% of all B-cell lymphomas and up to 50% of primary gastric lymphomas.¹⁶ The most common genetic alteration is t(11;18)(q21;q21), seen in about 50% of all cases. There is a strong association with autoimmune disorders such as Hashimoto thyroiditis, Sjögren syndrome, and *Helicobacter pylori*-associated chronic gastritis. Stomach, lung, salivary gland, skin, ocular adnexa, thyroid, and breast are commonly affected sites. Patients with MALT lymphoma usually have stage I or II disease, and are potentially curable by

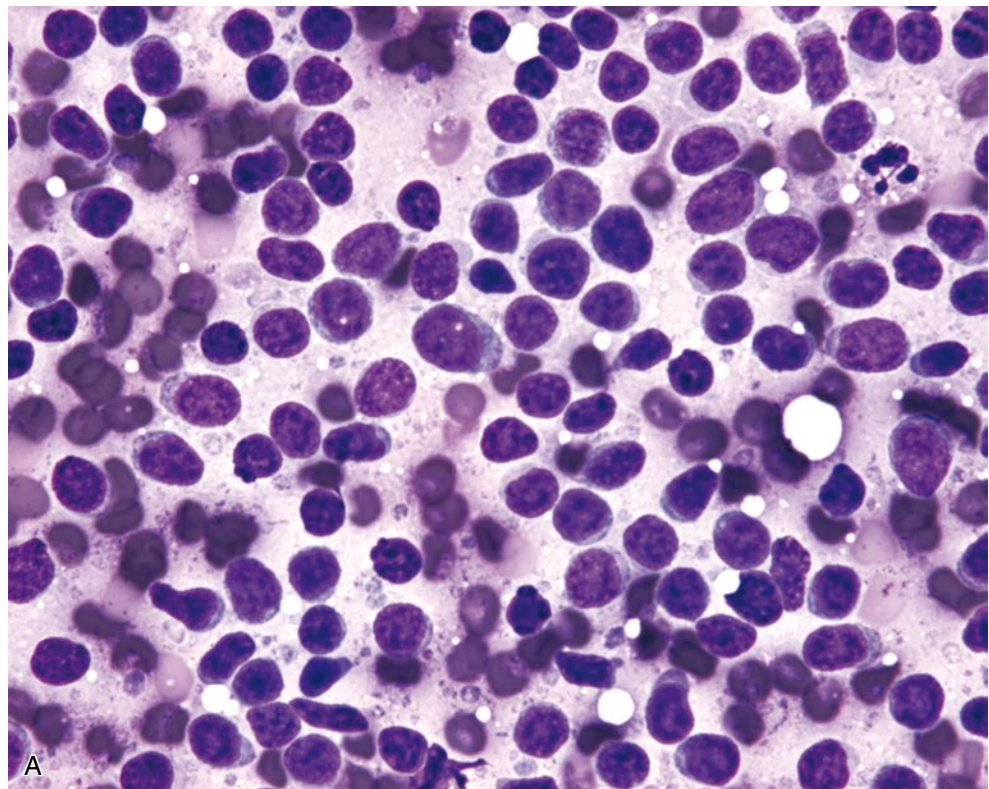


Figure 11.15 Mantle cell lymphoma (MCL). A, The smear shows an isomorphic population of small cells with round-to-oval nuclei and scant cytoplasm. Note the neutrophil at upper right for size comparison (Romanowsky stain). B, Fluorescence in situ hybridization (FISH) reveals an (11;14) translocation in the abnormal cells on the right. A green probe for immunoglobulin heavy chain (IgH) at 14q32 and a red probe for the cyclinD1 gene come together to give a yellow signal when there is a translocation. Note that the green and red signals are far apart in the normal cells. (Dual color interphase FISH. B, Courtesy of Paola dal Cin, PhD, Brigham and Women's Hospital, Boston.)

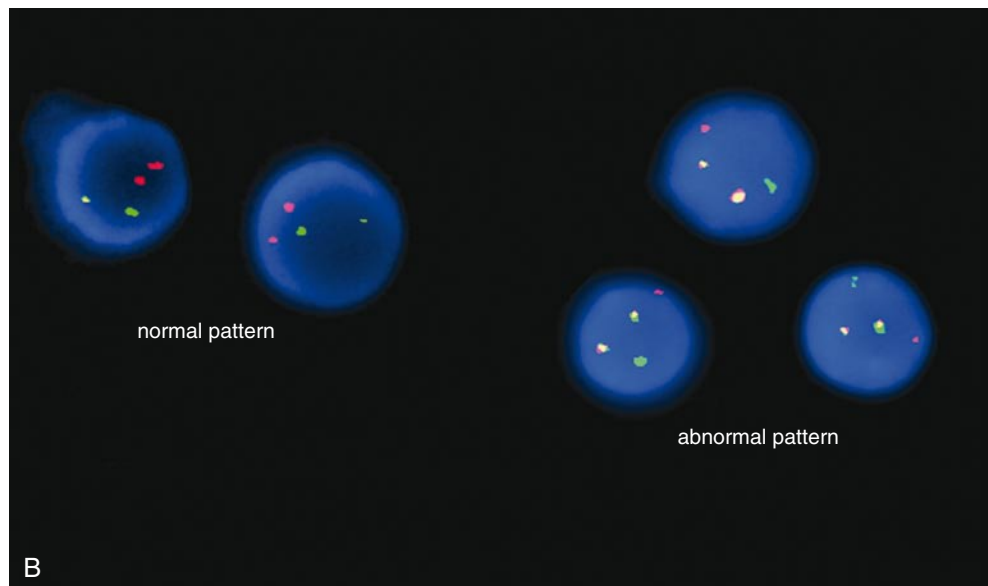


TABLE 11.6 DIFFERENTIAL IMMUNOPHENOTYPE AND GENETICS OF SMALL B-CELL LYMPHOMAS

	Small Lymphocytic	Mantle Cell	Follicular	Marginal Zone	Lymphoplasmacytic
CD5	+	+	–	–	–
CD10	–	–/+	+/-	–	–
CD20	+/-	+	+	+	+
CD23	+	–	–/+	–	–
cyclin D1	–	+	–	–	–
CD43	+	+	–	–/+	+/-
Tdt	–	–	–	–	–
genetics	trisomy 12 (30%), others	t(11;14)	t(14;18)	trisomy 3, t(11;18), others	inconsistent

+/-, Majority of cases are positive, small percentage negative.

–/+, Majority of cases are negative, small percentage positive.

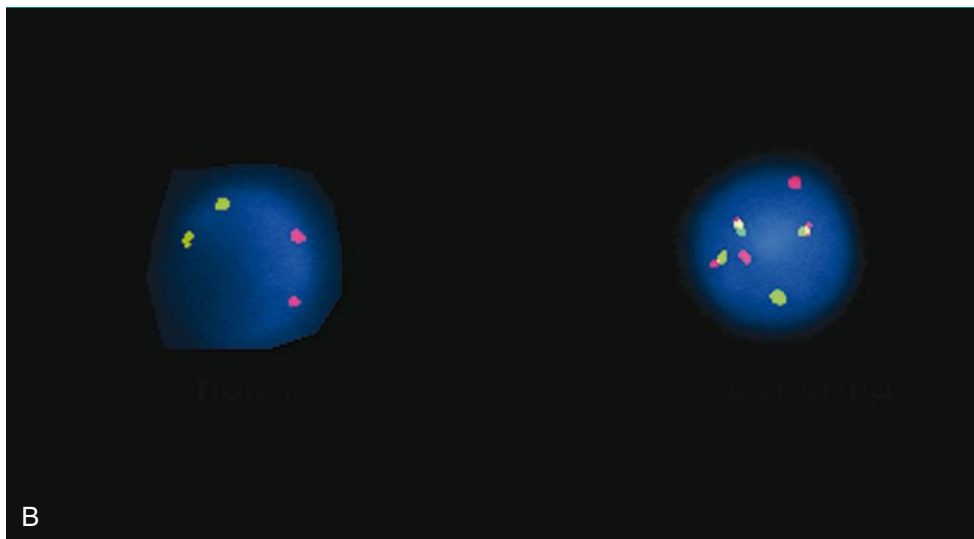
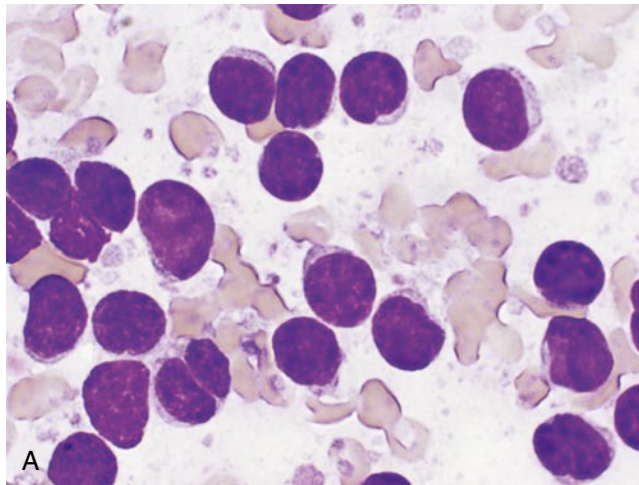


Figure 11.16 Follicular lymphoma (FL). A, Note the nuclear irregularities and occasional clefted nucleus. Lymphoglandular bodies are numerous (Romanowsky stain). B, Fluorescence in situ hybridization (FISH) reveals a (14;18) translocation in the abnormal cell on the right. A green probe for immunoglobulin heavy chain (IgH) at 14q32 and a red probe for bcl-2 at 18q21 come together to give a yellow signal when there is a translocation. Note that the green and red signals are far apart in the normal cell. (Dual color interphase FISH. B, Courtesy of Paola dal Cin, PhD, Brigham and Women's Hospital, Boston.)

surgery or regional radiotherapy. Antibiotic therapy for *Helicobacter* infection often results in remission of gastric MALT lymphoma.

Histologically, the neoplastic cells resemble small lymphocytes or centrocytes, some of which have more abundant cytoplasm, leading to a monocytoid appearance. Plasma cells are present in many cases. In glandular organs, nests of neoplastic cells invade the epithelium, resulting in characteristic “lymphoepithelial lesions.”

CYTOMORPHOLOGY OF MARGINAL ZONE LYMPHOMA:

- polymorphous population
- small-sized and intermediate-sized lymphocytes
- round or irregular nuclei
- “monocytoid” cells (moderate amount of pale cytoplasm)
- plasma cells
- follicular dendritic cells
- few tingible-body macrophages, lymphohistiocytic aggregates, immunoblasts

The heterogeneous cell population makes it difficult to recognize the lesion as a lymphoma. Unfortunately, the diagnostically useful lymphoepithelial lesions are difficult (or impossible) to appreciate in smears. A mixed population of small and intermediate lymphocytes with pale staining cytoplasm (monocytoid cells), large transformed cells, plasmacytoid cells, and plasma cells in sites where lymphoid tissue is normally sparse should raise the suspicion of MALT lymphoma⁹⁴ (Fig. 11.17). IP is critical to establish the diagnosis in most cases.

Lymphoplasmacytic Lymphoma. Lymphoplasmacytic lymphoma (LPL) is a rare B-cell malignancy (only 1.5% of nodal lymphomas). LPL occurs in older adults (mean age 63) and commonly involves the bone marrow and spleen in addition to lymph nodes. Most patients have a serum monoclonal immunoglobulin M (IgM) paraprotein. If levels exceed 3 g/dL, the patient is considered to have Waldenström macroglobulinemia, with potential complications of hyperviscosity (such as visual deficits and stroke). LPL is an indolent tumor, and asymptomatic patients are usually not treated. Transformation to DLBL occurs in a minority of patients.

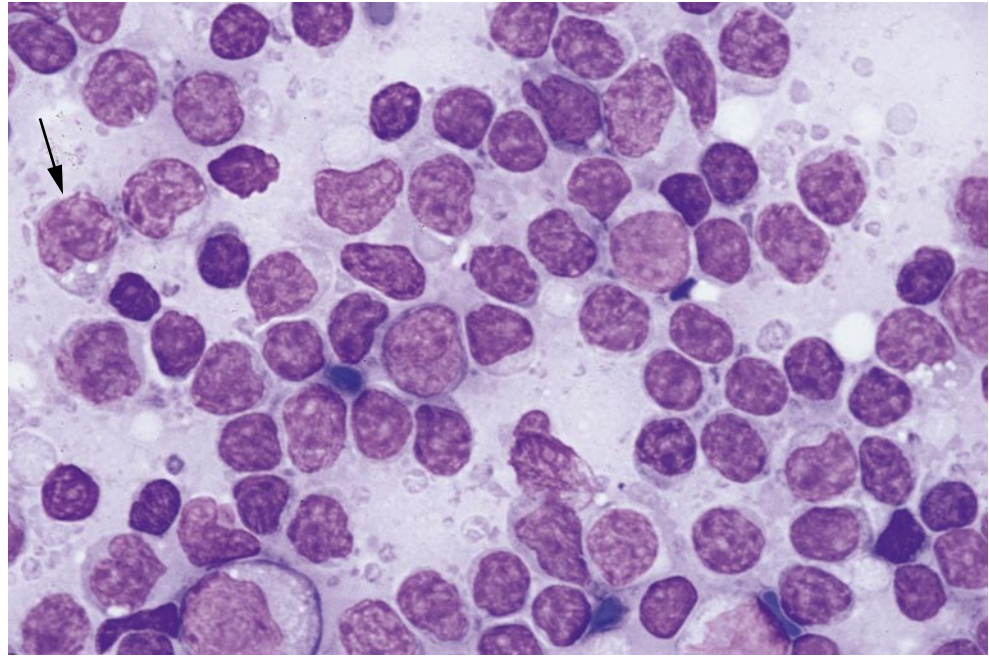


Figure 11.17 Marginal zone lymphoma (MZL). A mixture of small-sized and intermediate-sized lymphocytes have irregular nuclear contours, and occasional monocytoid cells are present (arrow). Lymphoglandular bodies are numerous (Romanowsky stain).

Histologically, LPL is a diffuse proliferation of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. Because other lymphomas (SLL, FL, MZL) can contain a similar mixture of cells, LPL is a diagnosis of exclusion. LPL cells express B-cell antigens CD19 and CD20 and are CD5–, CD23–, and CD10–.

CYTOMORPHOLOGY OF LYMPHOPLASMACYTIC LYMPHOMA:

- cellular specimen
- lymphocytes
- plasmacytoid lymphocytes
- plasma cells
- Dutcher bodies
- epithelioid histiocytes
- mast cells

Cytologic preparations are comprised of various proportions (from case to case) of small lymphocytes, plasma cells, and cells with intermediate features (“plasmacytoid lymphocytes”). In some cases, intranuclear immunoglobulin pseudoinclusions called Dutcher bodies are seen. Epithelioid histiocytes and mast cells are often present. The morphologic findings are not specific for LPL. SLL, FL, and MZL should be considered in the differential diagnosis and must be excluded before the diagnosis of LPL is made.

Differential diagnosis of small cell lymphomas. The differential diagnosis of the small cell lymphomas includes reactive hyperplasia, HL, metastatic small cell carcinoma, and each of the five small cell lymphomas themselves.

DIFFERENTIAL DIAGNOSIS OF SMALL CELL LYMPHOMAS:

- reactive hyperplasia
- HL
- small cell carcinoma
- SLL
- mantle cell lymphoma
- follicular lymphoma
- MZL or MALT lymphoma
- LPL

The five small cell lymphomas must, first and foremost, be distinguished from a *reactive hyperplasia*. There are helpful morphologic clues, but they are not perfect, and in practice, IP is commonly relied on to assist with this distinction (see [Table 11.6](#)). If a smear is comprised of a monomorphous population of small lymphoid cells, lymphoma is likely, but a reactive lymph node cannot be excluded without IP, because a minimally reactive lymph node can also show little lymphocyte polymorphism.⁹⁵ Although small round lymphocytes predominate in reactive hyperplasia, in most cases there is an expanded range of lymphoid cells, including plasmacytoid lymphocytes, plasma cells, and immunoblasts. Dendritic-lymphocytic aggregates and tingible-body macrophages, although common in reactive hyperplasia, are rare or absent in the small cell lymphomas, with the exception of FL.

MZL, in particular, is an exception to the rule of morphologic homogeneity in the small cell lymphomas. MZL resembles a reactive hyperplasia because of its polymorphous infiltrate of small lymphocytes, centrocytes, and

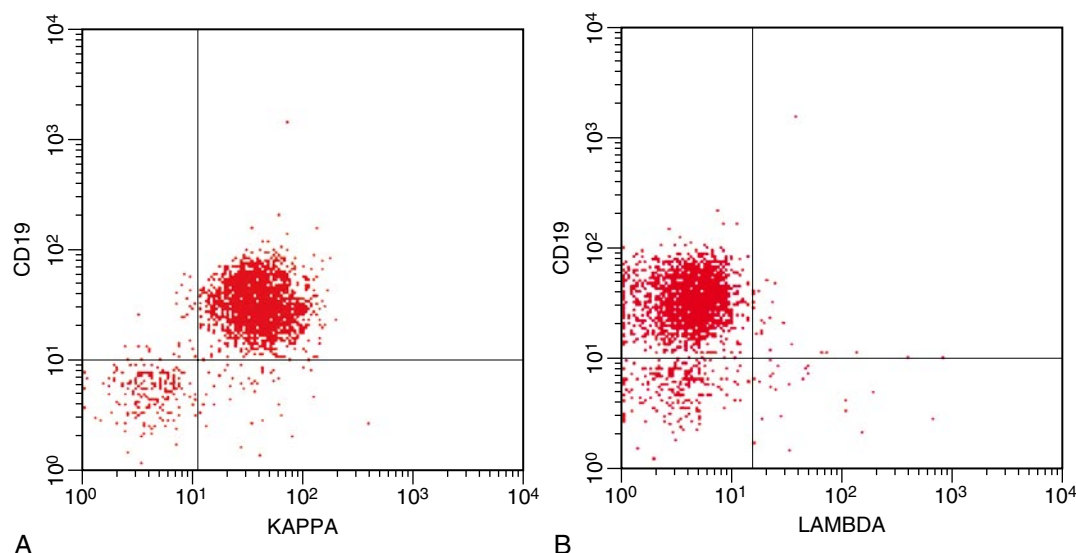


Figure 11.18 Non-Hodgkin lymphoma (NHL). Flow cytometric analysis shows a population of CD19-positive B cells that are positive for κ , A, and negative for λ light chains, B.

monocytoid B cells.¹² Ultimately, the distinction between lymphoma and reactive hyperplasia rests on IP, either by FCM (Fig. 11.18) or immunocytochemistry.

IP also helps in the distinction between NHL and a HL with few RS cells; the small B-cells of HL show polyclonal expression of immunoglobulin light chains.

The nonlymphoid malignancy in adults that most commonly mimics one of the small cell lymphomas is *small cell carcinoma*. Small cell carcinoma, like the lymphomas, yields highly cellular aspirates, but unlike them, contains prominent cell clusters with nuclear molding. It also characteristically shows extensive necrosis (Fig. 11.19) and occasional “paranuclear blue

bodies,” solitary round, light to dark blue, homogeneous spheres that can indent the nucleus.⁹⁶ Abundant nuclear and cytoplasmic debris is rare in all of the small cell lymphomas. This is particularly helpful in the occasional case of small cell carcinoma in which cell clusters are rare. Necrosis should not be confused with lymphoglandular bodies, which do not contain nuclear fragments.

The small cell lymphomas are distinguished from one another by a combination of clinical, morphologic, immunophenotypic, and genetic features. Clinical features are summarized in Table 11.5. An extranodal location is common in MZL and MCL and may be seen in FL (particularly

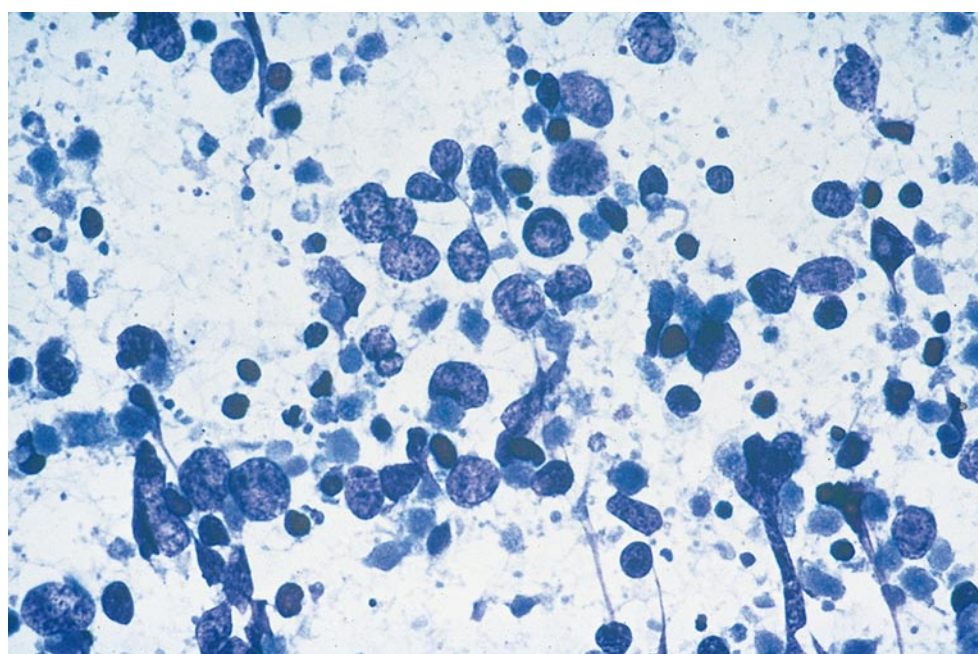


Figure 11.19 Small cell carcinoma. When cell clusters are sparse and there is smearing of cell nuclei in a background of necrotic debris, the smears mimic lymphoma. Fragments of cytoplasm from necrotic cell “ghosts” can be misinterpreted as lymphoglandular bodies (Papanicolaou stain).

in the head and neck region), but it is rarely the presenting site of SLL. Clinical evidence of Waldenström macroglobulinemia is highly associated with LPL.

Morphologically, SLL and MCL are more monomorphous than FL, MZL, and LPL. In particular, SLL is composed of uniform small round cells, with relatively infrequent larger cells. MCL is also composed of monomorphous small cells, with more finely textured chromatin than SLL. Monocytoid B cells are a clue to the diagnosis of MZL, and notched and grooved nuclei are characteristic of follicular lymphoma. An infiltrate that includes plasma cells and plasmacytoid cells is characteristic but not diagnostic of LPL. Although morphologic and clinical features are helpful, the immunophenotype, particularly the expression of CD5, CD10, and CD23, is paramount in subclassifying the small cell lymphomas.

Algorithm for discriminating among small cell lymphomas based on immunophenotype:

- CD5+ (SLL and MCL)
 - CD23+ = SLL
 - CD23– = MCL
 - light chain, CD20 dim = favor SLL
- CD5– (FL, MZL, LPL)
 - CD10+ = FL
 - CD10– = unresolved

The CD5+, CD10–, CD23+ immunophenotype is highly specific for SLL (Fig. 11.20), and the CD5+, CD10–, CD23– immunophenotype is highly specific for MCL (Fig. 11.21). With SLLs, fluorescence for light chains and

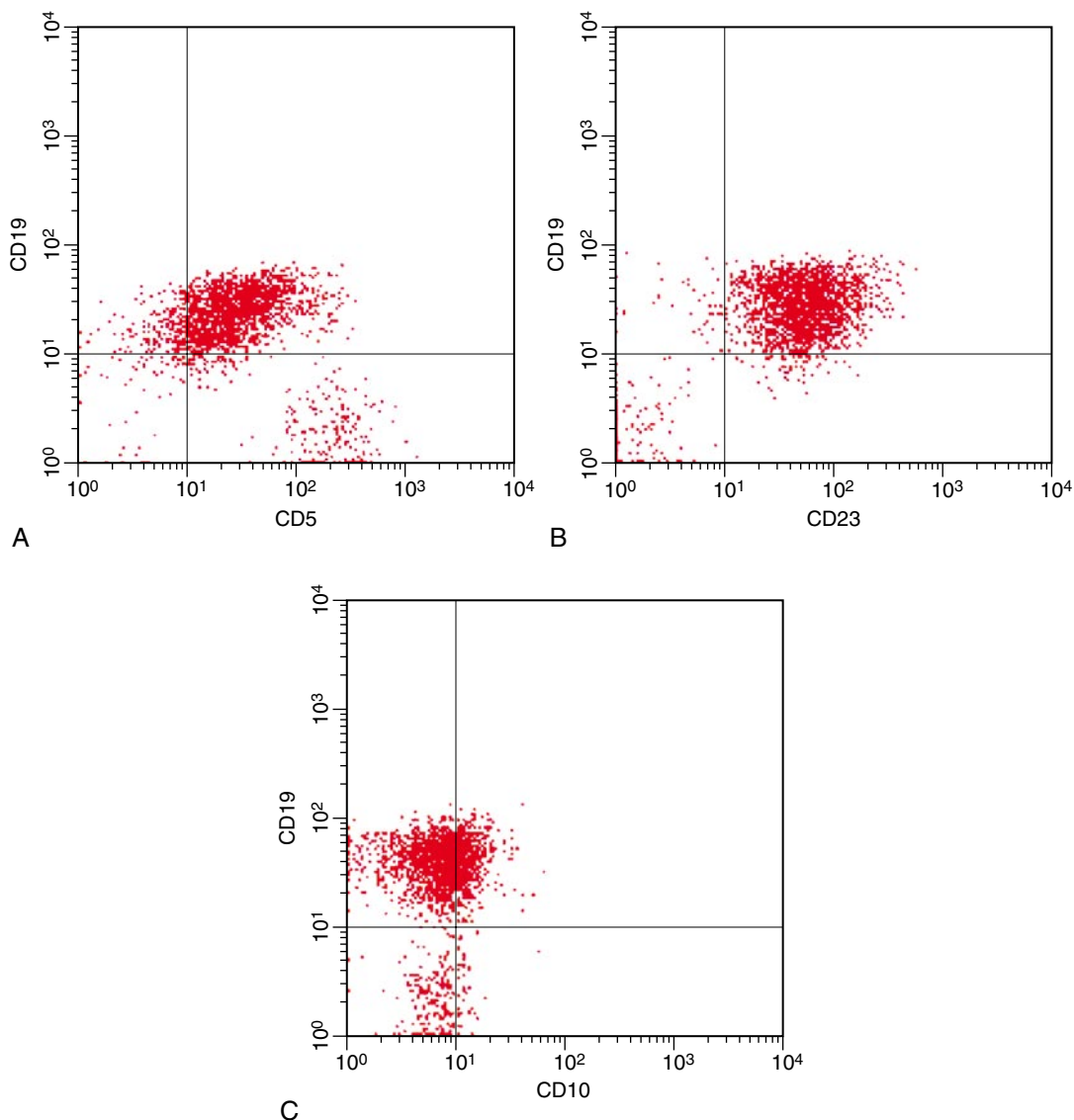


Figure 11.20 Small lymphocytic lymphoma (SLL). Flow cytometric analysis shows a population of CD19-positive B cells that are A, positive for CD5, B, positive for CD23, and C, negative for CD10.

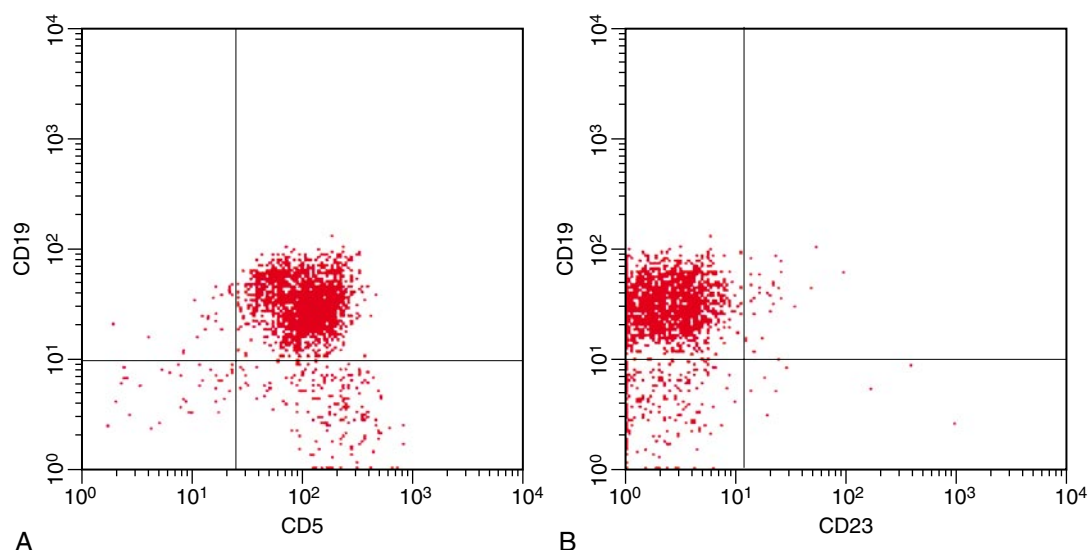


Figure 11.21 Mantle cell lymphoma (MCL). Flow cytometric analysis shows a population of CD19-positive B cells that are A, positive for CD5 and B, negative for CD23. The malignant cells were also negative for CD10 (not shown).

CD20 is characteristically weak (commonly referred to as “dim”). In addition, increased cyclin D1 (bcl-1) expression, which can be measured by immunocytochemistry or in situ hybridization, is highly specific for MCL. The CD5⁺, CD10⁺ immunophenotype (Fig. 11.22) is characteristic of FL (CD23 can be + or –), but many cases of FL are negative for CD10. The CD5⁺, CD10[–] profile is therefore non-specific because it does not differentiate among FL, MZL, and LPL; in such cases an open biopsy might be needed.

Aberrant immunophenotypes occur, therefore the immunophenotype must be correlated with morphologic, clinical, and where appropriate, molecular genetic

studies. Molecular genetic studies are indeed helpful (see Table 11.6), particularly when the immunophenotype is aberrant or ambiguous. About 30% of SLLs show trisomy 12. The t(11;14)(q13;q32) translocation is a hallmark of MCL and is detected in most cases. FL is characterized by a t(14;18)(q32;q21) translocation in up to 95% of cases. These characteristic abnormalities can be identified by FISH on cytologic preparations.^{38,85,86}

Note that the distinction between grade 3 FL and DLBL by FNA is not possible because of the loss of tissue architecture.¹² The Ki-67 proliferation marker and centroblast counting may be helpful in overcoming this problem.^{92,93} Of note, tumors that have a vastly

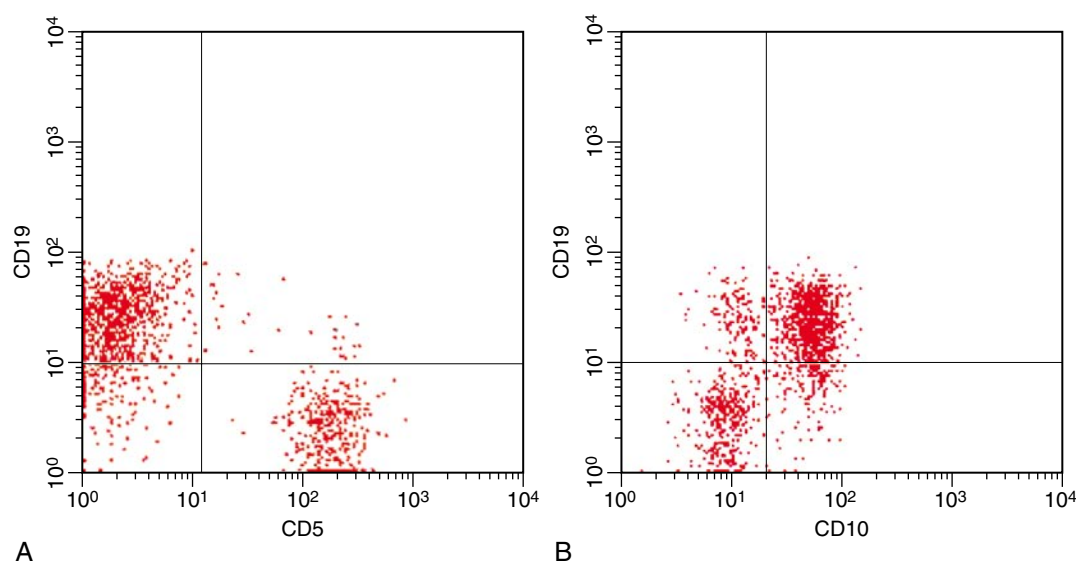


Figure 11.22 Follicular lymphoma (FL). Flow cytometric analysis shows a population of CD19-positive B cells that are A, negative for CD5. Note that there is a population of CD19-negative T cells that expresses CD5. The CD19-positive B cells are also positive for CD10, B.

predominant population of centroblasts (i.e., grade 3 FL) are generally treated like DLBL; thus determination of follicular versus diffuse architecture may not be critical in such cases.

Lymphomas of Large Cells

A broad and heterogeneous group of neoplasms that arise from B cells, T cells, or natural killer (NK) cells, this category is nonetheless dominated by a single common entity—DLBL. Unlike the previous group of small B-cell lymphomas, which are tumors of adults, many of the large cell lymphomas occur in children and adults. In children, the most common lymphomas are DLBL, lymphoblastic lymphoma, and Burkitt lymphoma.⁹⁷

Note that the designation large cells for the entities described in this section is used here as a convenience. Although some of these neoplasms are indeed comprised predominantly of large cells, others contain a mixture of small and large neoplastic cells, or large neoplastic cells with reactive (nonlesional) small cells. Some are actually comprised of intermediate-sized cells (e.g., Burkitt lymphoma). Lymphoblastic lymphoma cells are often smaller cells, but it is considered here under the broader heading of T-cell lymphomas. Finally, not all post-transplant lymphoproliferative disorders are even lymphomatous, but they are discussed here because most of them are neoplastic, and those that are neoplastic usually contain at least some, if not a preponderance of, large cells. The differential diagnosis of the lymphomas of large cells is discussed in a dedicated section after the descriptions of the individual entities.

Diffuse Large B-Cell Lymphoma. DLBL is an aggressive but potentially curable subtype of NHL that comprises 35% of adult lymphomas. It is also one of the three major lymphomas in children. About 40% of patients present with extranodal disease. Several clinically distinct variants of DLBL are worth noting. Primary DLBL of the mediastinum predominates in young adult females and may cause superior vena cava obstruction. FNA has been used successfully in diagnosing this variant,^{34,98} but the sclerosis may limit FNA sampling to a few inconspicuous cells. Two other variants are primary effusion large B-cell lymphoma (see Chapter 4) and intravascular large B-cell lymphoma (see Chapter 14).

The histologic hallmark of DLBL is the presence of confluent areas of large malignant B cells. Several morphologic subtypes have been recognized (centroblastic, immunoblastic, T-cell or histiocyte rich, anaplastic large B-cell, and others) but subclassification is not clinically relevant.⁹⁹ Through gene expression profiling, different genetic subtypes of DLBL—germinal center B-cell-like, activated B-cell-like, and type 3—have been identified.¹⁰⁰ These have clinical significance in that they predict survival after chemotherapy.¹⁰¹ Preliminary evidence suggests that gene expression profiling can be performed on FNAs.⁴¹

The bcl-2 gene rearrangement is found in 30% of cases.⁹⁰ Another 30% show abnormalities of the 3q27 region involving bcl-6.

CYTOMORPHOLOGY OF DIFFUSE LARGE B-CELL LYMPHOMA:

- predominantly large cells (2.5 to 5 times the size of a small lymphocyte). Subtypes:
 - centroblastic
 - immunoblastic
 - background rich in small lymphocytes (T cells) or histiocytes
 - anaplastic
- variable number of tingible-body macrophages
- paucity or absence of dendritic-lymphocytic aggregates

The cellularity of smears from DLBL is variable. Aspirates of mediastinal DLBL can be sparsely cellular as a result of the extensive sclerosis common to these tumors. Most FNAs of DLBL are at least moderately cellular, however, and smears are usually easily recognizable as abnormal because of the abundance of large atypical cells (Fig. 11.23). They can have a round or highly irregular nucleus with multiple nucleoli and scant cytoplasm (centroblastic type); a round or irregular nucleus with a single prominent nucleolus and more abundant cytoplasm (immunoblastic type); or a bizarre, pleomorphic nucleus with frequent multinucleation that resembles the nucleus of RS cells (anaplastic type). A population of small, non-neoplastic T-cells is always present but usually represents a minority of the cell sample. In some cases, however, small T-cells, accompanied by histiocytes, outnumber the large, neoplastic B-cells (T-cell or histiocyte rich type).

Characterization of DLBL by FCM can be challenging, both because the large, atypical cells may appear outside the usual “lymphoid gate” on FCM scattergrams and because the cells may not survive processing as a result of fragility.¹⁰² Determination of clonality by PCR may be required in these instances.

Burkitt Lymphoma. Burkitt lymphoma (BL) is a highly aggressive but potentially curable B-cell malignancy with a characteristic morphology and a cytogenetic translocation that constitutively activates the c-myc oncogene. The diagnosis of BL has great clinical importance because it may necessitate a chemotherapeutic regimen that is more aggressive than that used for other high-grade B-cell lymphomas.

BL occurs in three clinical settings: (1) an *endemic* form found in Africa and Asia and rare in the United States and Europe; (2) a sporadic form, which occurs in the United States and other countries; and (3) an *immunodeficiency-associated* form. The endemic and sporadic forms are more common in children, and the

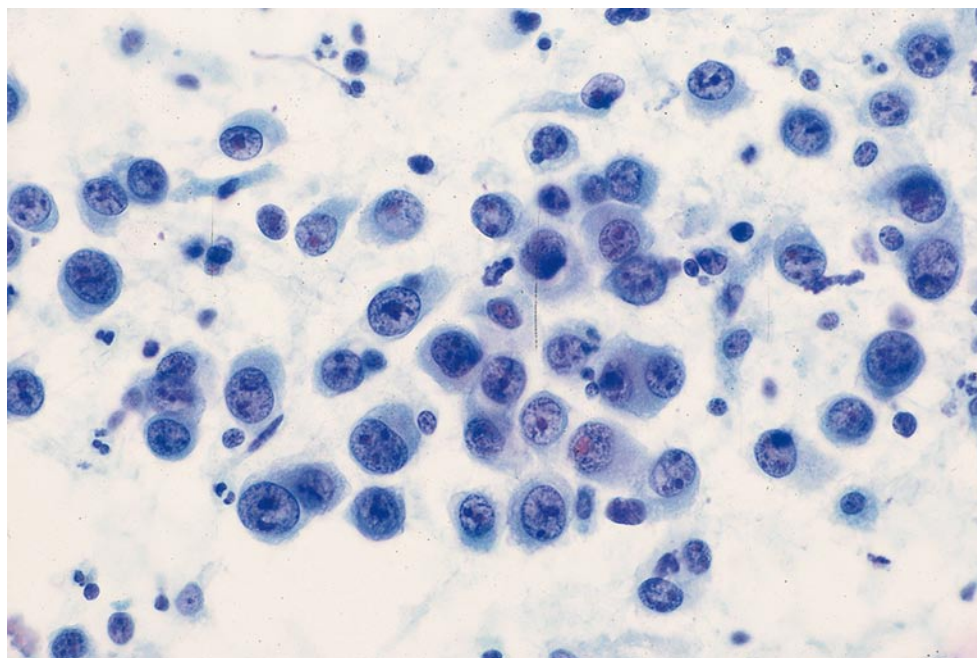


Figure 11.23 Diffuse large B-cell lymphoma (DLBL). A uniform population of lymphocytes with large, almost vesicular nuclei, obvious nucleoli and a moderate amount of cytoplasm is mixed with a background of neutrophils and some small lymphocytes (Papanicolaou stain).

immunodeficiency-associated form is more common in adults. Virtually all the endemic cases show latent infection of the tumor cells by EBV, whereas only a subset of the sporadic and immunodeficiency-associated cases is EBV associated.

The malignant cells involve lymph nodes or extranodal tissues (the gastrointestinal tract, liver, and bone marrow are common sites in the nonendemic form), and cerebrospinal fluid is involved in 20% to 30% of cases at presentation.

CYTOMORPHOLOGY OF BURKITT LYMPHOMA:

- uniform intermediate-sized cells
- round nuclei, coarse chromatin
- conspicuous 2 to 5 nucleoli per nucleus
- scant blue cytoplasm with small vacuoles (Wright-Giemsa)
- apoptosis and numerous mitoses
- numerous tingible-body macrophages

Smears are monotonously hypercellular. Tingible-body macrophages are randomly dispersed throughout the smear, mimicking the “starry sky” pattern of tissue sections. Because individual cell necrosis (apoptosis) is common, a “dirty” background is typical. The cells are intermediate in size, with round nuclei that have coarse chromatin, multiple (but small) nucleoli, and scant cytoplasm (Fig. 11.24a). With Romanowsky-type stains, the cytoplasm is a deep blue with small lipid vacuoles.¹⁰³ These cytoplasmic features are not specific for BL, however, and can be seen in DLBL.

BL cells express B-cell-specific surface antigens such as CD19, CD20, the immunoglobulin heavy chain M, and monotypic surface immunoglobulin light chains. They also express CD10.

BL is invariably associated with a chromosomal translocation involving the *c-myc* proto-oncogene on chromosome 8 and either the immunoglobulin G (IgG) heavy chain or κ or λ light chain genes.

The three most common translocations and their frequencies in Burkitt lymphoma:

- t (8;14): 80%
- t (2;8): 15%
- t (8;22): 5%

The most reliable method for identifying the specific translocation in BL is karyotyping by conventional cytogenetics. The presence of any of these three translocations can also be documented by FISH.^{104,105} Using probes to the flanking regions of the *c-myc* locus, a split signal indicates a rearrangement (Fig. 11.24B).

A *c-myc* rearrangement is highly sensitive but not specific for BL because it is occasionally seen in large B-cell lymphoma, rare cases of follicular lymphomas, and some late-stage multiple myelomas. For this reason, the diagnosis is based on an integration of clinical, morphologic, immunophenotypic, and genetic findings.

T-cell Lymphomas. T-cell lymphomas comprise about 10% of all NHL in Western countries. The WHO classification lists numerous subtypes (Table 11.7) that are

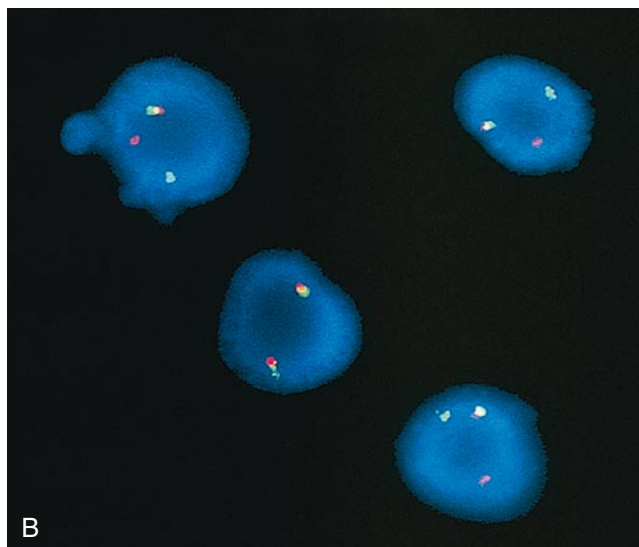
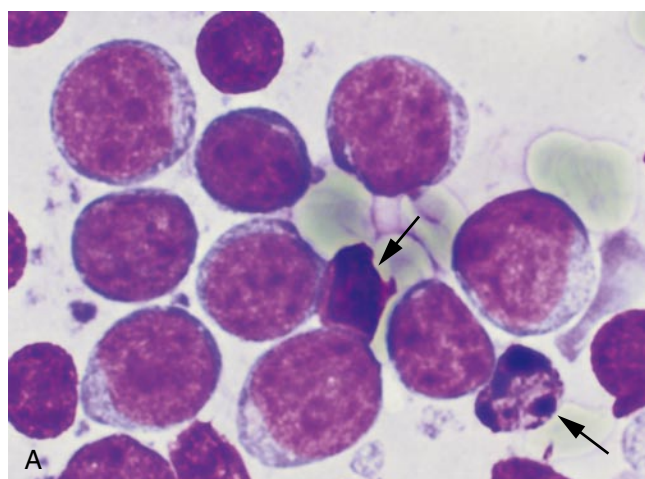


Figure 11.24 Burkitt lymphoma (BL). A, The intermediate-sized neoplastic lymphocytes have round nuclei, multiple nucleoli, blue cytoplasm, and small cytoplasmic vacuoles. Apoptotic bodies (arrows) are present (Romanowsky stain). B, Detection of c-myc translocation by fluorescence in situ hybridization (FISH). Red and green fluorescent probes to regions flanking c-myc are split apart. This occurs in all three of the common c-myc translocations. (Dual color interphase FISH. B, Courtesy of Paola dal Cin, PhD, Brigham and Women's Hospital, Boston.)

TABLE 11.7 WORLD HEALTH ORGANIZATION'S CLASSIFICATION OF T- AND NK-CELL NEOPLASMS*

Precursor T-cell lymphoblastic leukemia or lymphoma

Mature T-cell and NK-cell neoplasms

T-cell prolymphocytic leukemia

T-cell large granular lymphocytic leukemia

Aggressive NK-cell leukemia

Extranodal NK/T-cell lymphoma, nasal-type

Mycosis fungoides

Sézary syndrome

Angioimmunoblastic T-cell lymphoma

Peripheral T-cell lymphoma, unspecified

Adult T-cell leukemia/lymphoma (human T-cell leukemia virus 1 positive)

Anaplastic large cell lymphoma (T-cell and null cell types)

Primary cutaneous anaplastic large cell lymphoma

Subcutaneous panniculitis-like T-cell lymphoma

Enteropathy-type T-cell lymphoma

Hepatosplenic T-cell lymphoma

*More common entities are in bold.

broadly divided into precursor T-cell lymphoblastic lymphoma and mature T-cell and NK-cell lymphomas.

The mature T-cell and NK-cell neoplasms comprise a diverse group of neoplasms that are defined primarily by their clinical features. In contrast to B-cell lymphomas, most T-cell lymphomas are not associated with specific immunophenotypic profiles. With the exception of anaplastic large cell lymphoma, which is sometimes associated with the t(2;5) translocation, specific genetic abnormalities have not been identified for many T-cell and NK-cell neoplasms.

Unlike B-cell lymphomas, in which light chain restriction is a marker of malignancy, there are no convenient

antigenic markers of monoclonality. The diagnosis is suggested by an aberrant T-cell immunophenotype, such as loss of one or more of the pan T-cell markers CD2, CD3, CD5, CD7; loss of both CD4 and CD8; or coexpression of both CD4 and CD8.³² Care must be taken, however, not to base the diagnosis of T-cell lymphoma solely on the presence of a double positive (CD4+CD8+) T-cell population, which can be seen in nodular lymphocyte predominant HL.⁵⁶ Molecular genetic studies, most commonly PCR for rearrangement of the T-cell receptor genes, are often required to confirm the clonality of a T-cell proliferation.⁵⁸

Peripheral T-cell lymphoma unspecified and anaplastic large cell lymphoma are the most common mature T-cell lymphomas.

Peripheral T-cell lymphoma, unspecified. Peripheral T-cell lymphoma of unspecified type is much more common in Asia than Europe or North America. In the United States, these patients are typically elderly and systemically ill, with fevers, night sweats, and bulky lymphadenopathy.

CYTOMORPHOLOGY OF PERIPHERAL T-CELL LYMPHOMA, UNSPECIFIED:

- monomorphous small or large lymphocytes, or a mixture of small and large lymphocytes
- irregular nuclei
- histiocytes, plasma cells, and eosinophils
- RS-like cells

Peripheral T-cell lymphoma cases show marked variety in cell composition. Most aspirates (70%) have

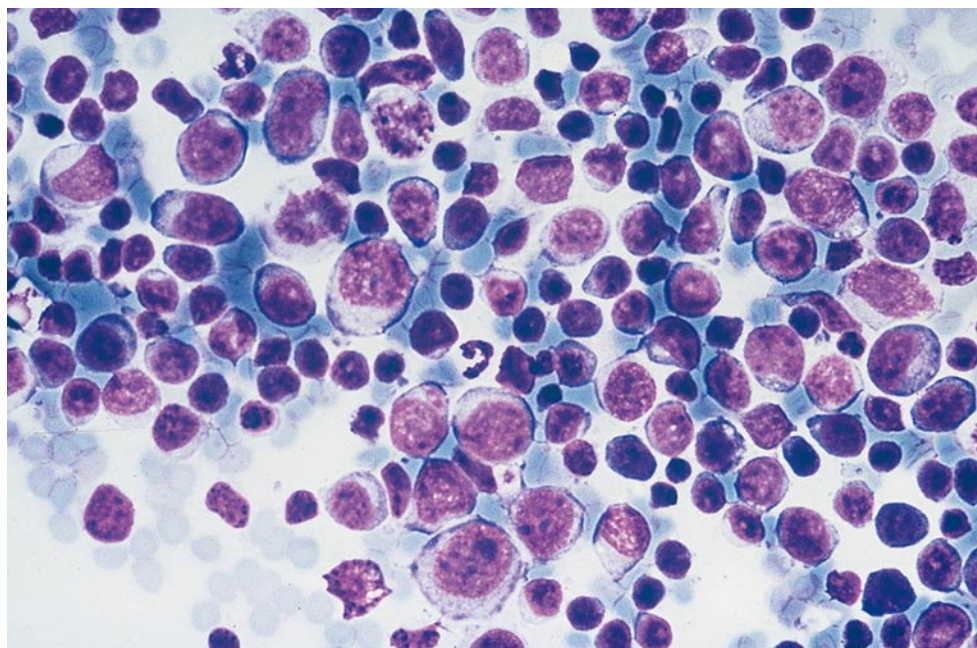


Figure 11.25 Peripheral T-cell lymphoma, unspecified. A picture of lymphocyte heterogeneity is produced by this mixture of small, intermediate, and large cells. In other fields of the smear the large cells were more numerous, but taken in isolation this image is easily misinterpreted as representing reactive hyperplasia (Romanowsky stain).

a polymorphous cell population with histiocytes, eosinophils, plasma cells and intermediate and large lymphocytes (Fig. 11.25), whereas others have the morphology of a pure large cell lymphoma, and a smaller number have a monomorphic SLL appearance.^{28,32,106} Nuclei of both small and large lymphocytes frequently display nuclear indentations, grooves, and knoblike projections.

Anaplastic large cell lymphoma. Anaplastic large cell lymphoma (ALCL) is a subtype of NHL that was recognized after the discovery of the CD30 (Ki-1) antigen. In the past, many of these were misdiagnosed as carcinomas and other nonlymphoid malignancies, or as undifferentiated malignant neoplasms. Initially they were called Ki-1 lymphomas, but Ki-1 (CD30) expression is not limited to ALCL.

ALCL accounts for about 3% of adult NHL and 10% to 30% of childhood lymphomas.¹⁶ It is a T-cell neoplasm and usually expresses one or more T-cell antigens. Some cases have an apparent null cell phenotype, but their T-cell lineage is revealed by demonstrating clonal T-cell receptor rearrangement.

They show a broad morphologic spectrum, but in the common type, the cells are large and pleomorphic. Histologically, a bizarre cell with a horseshoe-shaped nucleus (the so-called “hallmark cell”) is characteristic. Reflecting the fact that lymph nodes are often only partially involved (with a sinusoidal growth pattern in histologic sections), the FNA sample can contain a mixed lymphoid background that obscures the hallmark cells. In addition to this classic form, there is a *lymphohistiocytic variant*, characterized by a large number of histiocytes that may mask the malignant cells, and a *small cell variant*, which resembles a peripheral T-cell lymphoma.

Some cases of ALCL are associated with a t(2;5)(p23;q35) translocation, which fuses the ALK gene with the nucleophosmin (NPM) gene to produce the ALK protein, but many of these tumors in adults lack this translocation, and ALK expression is detected in 60% to 85% of cases. Immunostaining for the ALK protein is helpful both in diagnosis and prognosis; patients with the t(2;5) or ALK positivity have a better prognosis than patients who are ALK negative.¹⁰⁷

CYTOMORPHOLOGY OF ANAPLASTIC LARGE CELL LYMPHOMA:

- intermediate and large cells
- irregular nuclei: horseshoe, donut, and embryoid shapes
- RS-like cells
- histiocytes, neutrophils
- no tingible-body macrophages or dendritic-lymphocytic aggregates
- few or absent lymphoglandular bodies

Aspirates show moderate to high cellularity; necrosis and inflammation can be prominent. The hallmark cell of ALCL has a horseshoe-shaped nucleus, but a spectrum of abnormal large cells varying in number, shape, and lobation of nuclei, particularly ring-shaped (donut) nuclei, are seen¹⁰⁸⁻¹¹⁰ (Fig. 11.26). Necrosis can be a prominent feature.¹¹¹ The tumor cells are positive for CD30 in a membranous pattern and in the Golgi region. Many cases are positive for ALK, EMA, and clusterin, but negative results do not exclude the diagnosis. EBV RNA sequences are absent. A break-apart FISH probe permits identification of the t(2;5) translocation in FNAs,¹¹² but

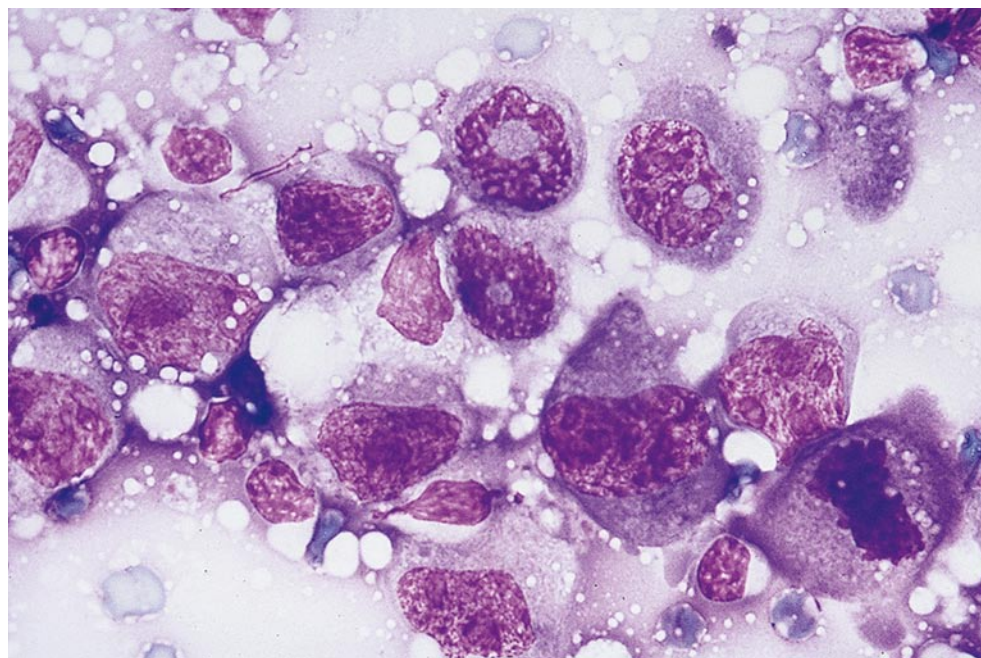


Figure 11.26 Anaplastic large cell lymphoma (ALCL). Large cells with marked variation in nuclear shape including “donut” forms are seen (Romanowsky stain).

confirmation of a T-cell receptor gene rearrangement may be necessary in more difficult cases.

Precursor T- and B-cell lymphoblastic lymphoma.

Lymphoblastic lymphoma (LL) is an aggressive lymphoma that comprises almost one half of childhood NHL and is more common in males. Lymphadenopathy almost always occurs above the diaphragm. An anterior mediastinal mass is commonplace (up to 80% of patients) and can induce clinical symptoms mimicking bronchial asthma (resulting from tracheal compression) or the superior vena cava syndrome. Because these patients often have a palpable neck mass and are critically ill (many with some degree of respiratory compromise), the expediency of FNA makes it the procedure of choice to establish the diagnosis. Combined with immunophenotypic studies, FNA has near perfect accuracy.^{21,22,113}

CYTOMORPHOLOGY OF LYMPHOBLASTIC LYMPHOMA:

- Lymphoblasts:
 - round or convoluted nuclei
 - finely granular chromatin
 - inconspicuous nucleoli
 - scant or moderate amount of cytoplasm

Smears are nearly always cellular and contain monotonous lymphoblasts to the near exclusion of any other cell type. Blasts are twice the size of a small lymphocyte, and nuclei are round or irregular. Nucleoli are either absent or inconspicuous (Fig. 11.27). Cytoplasm is usually scant and may contain tiny vacuoles. Mitotic figures are variable and depend on smear thickness. Tingible-body macrophages are variably present. About 90% of

cases are of T-cell derivation, the remainder derives from B-cells. In nearly all cases, blasts are positive for Tdt, a nuclear enzyme. IP, coupled with cell morphology and clinical features, is nearly always diagnostic. The clinical scenario usually aids in distinction from thymoma, an important clinical and immunophenotypic mimic that features lymphocytes with an “immature” immunophenotype (but typically mature cytomorphology). The epithelial component of a thymoma is often inconspicuous on FNA smears, requiring immunocytochemistry (for keratin) for confident identification.

Post-Transplant Lymphoproliferative Disorders. Post-transplant lymphoproliferative disorders (PTLDs) are a heterogeneous group of lesions that include both non-neoplastic lymphoid proliferations and a variety of lymphomas. They occur in about 1% to 2% of solid organ (e.g., kidney, liver, lung) and bone marrow transplant recipients.¹⁶ Eighty percent of PTLDs are associated with EBV infection and represent EBV-induced monoclonal or (less often) polyclonal B-cell proliferations; a small percentage are T-cell proliferations. PTLDs usually occur within the first year of transplantation, but they can also occur many years later. PTLDs tend to involve lymph nodes and the gastrointestinal tract, but other extranodal sites can also be involved, including the allograft itself. A significant proportion of PTLDs resolve after reduction of immune suppression. Some fail to regress, however, and are treated with cytotoxic chemotherapy.

Histologically, PTLDs are divided into four major categories.¹⁶ (1) “Early” lesions are polyclonal, non-neoplastic lymphoid proliferations that preserve the underlying lymph node architecture and show plasma cell hyperplasia or an infectious mononucleosis-like picture.

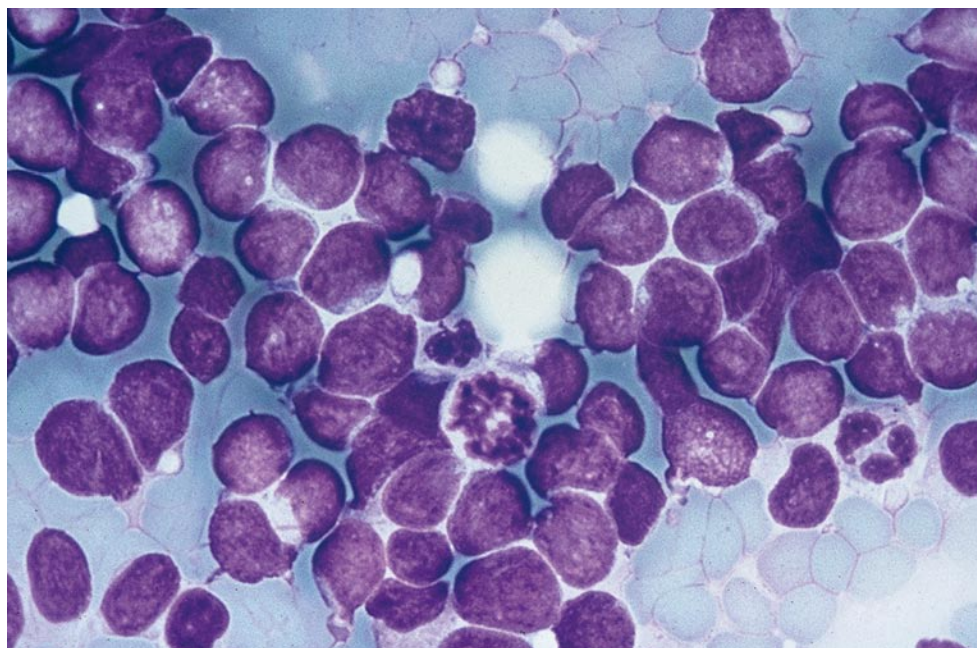


Figure 11.27 Lymphoblastic lymphoma (LPL). A pure population of blasts is present with finely dispersed chromatin and nuclear molding. A mitotic figure is seen. Compare the size of the blasts with the neutrophil at the lower right (Romanowsky stain).

(2) *Polymorphic PTLD* is a destructive, neoplastic proliferation that shows the full range of B-cell maturation, including immunoblasts, plasma cells, centrocytes, and small-sized and medium-sized lymphocytes. IP by FCM may appear polytypic, but molecular tests reveal clonal rearrangements of the immunoglobulin heavy chains and monoclonal episomal EBV. (3) *Monomorphic PTLD* is also a destructive, neoplastic proliferation, but it is composed of large, cytologically malignant lymphoid cells with prominent nucleoli resembling NHL. Most monomorphic PTLDs are B-cell neoplasms like DLBL, but some arise from T-cells. (4.) *HL and Hodgkin-lymphoma-like PTLD*.

The cytologic features mirror the varied histology of PTLD.⁴⁷ Early lesions and polymorphic PTLD have overlapping features cytologically (a mixture of immunoblasts, plasma cells, centrocytes, and small-sized and medium-sized lymphocytes) (Fig. 11.28A) but can be distinguished by molecular studies for clonality. The monomorphic PTLDs are cytologically malignant and are classified according to the WHO classification system of nontransplant-related lymphomas, but the term *PTLD* should be included in the diagnosis. The PTLD moniker is important because it prompts the oncologist to include reduction of immunosuppression in the therapeutic armamentarium.

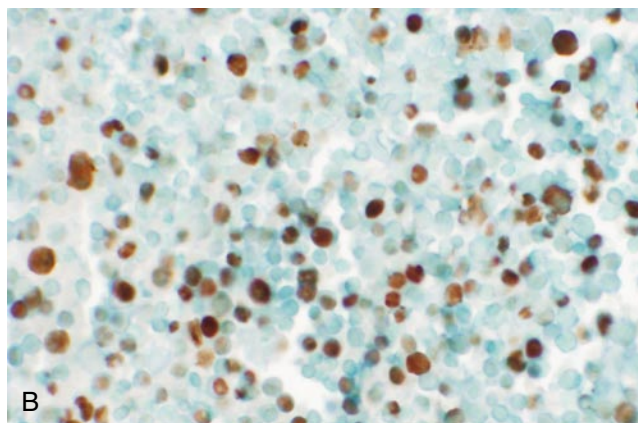
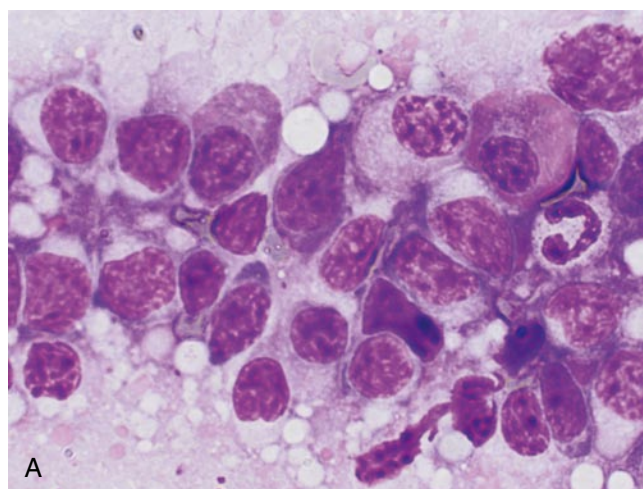


Figure 11.28 Post-transplant lymphoproliferative disorder (PTLD), polymorphic type. A, The smear shows a polymorphic population of small lymphocytes, plasmacytoid lymphocytes, plasma cells, and centrocytes (Romanowsky stain). B, In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA (EBER), performed on cell block sections, shows a positive reaction in many of the lesional cells.

Light chain restriction is sometimes absent by FCM or immunocytochemistry in polymorphic and monomorphic PTLDs; molecular testing is often needed to demonstrate clonality. Thus, the absence of light chain restriction does not exclude the diagnosis of PTLT. In situ hybridization for EBER can be quite helpful; a positive result for EBER in a majority of the lesional cells provides strong support for the diagnosis of PTLT⁴⁷ (Fig. 11.28B). About 20% of PTLTs are EBV-negative, however. In patients who received a bone marrow transplant for lymphoma, the differential diagnosis includes recurrent lymphoma. A positive result for EBER in a majority of the lesional cells helps establish the diagnosis of PTLT in this setting.

Differential Diagnosis of Large Cell Lymphomas

DIFFERENTIAL DIAGNOSIS OF LARGE CELL LYMPHOMAS:

- nonhematopoietic tumors
- reactive hyperplasia
- HL
- DLBL
- peripheral T-cell lymphoma
- ALCL
- precursor T- and B-cell lymphoblastic lymphoma
- thymoma
- BL and Burkitt-like lymphoma
- PTLT
- myeloid sarcoma (synonymous with granulocytic sarcoma and chloroma)
- histiocytic and dendritic cell neoplasms

The group of large cell lymphomas can mimic and be mimicked by *non-hematopoietic neoplasms*. Poorly differentiated large cell carcinomas, epithelioid sarcomas, melanoma, and germ cell tumors all can resemble DLBL. These nonlymphoid neoplasms usually show some degree of cell aggregation, which is highly unusual in DLBL. With rare exceptions (nasopharyngeal carcinoma and seminoma), the nonlymphoid tumors lack lymphoglandular bodies.

Conversely, ALCL is carcinoma-like.

Anaplastic large cell lymphoma is carcinoma-like because of:

- a paucity of lymphoglandular bodies¹¹¹
- cell clustering
- marked pleomorphism
- spindle cells¹¹⁴
- immunoreactivity for EMA
- scant smaller lymphocytes

Immunostains are particularly useful in the distinction between lymphoma and a nonhematopoietic neoplasm,

particularly keratins, S-100 protein, HMB-45, MelanA, OCT3/4 (for germ cell tumors), CD45, CD3, CD20, and CD30 (Ki-1). Of note, some ALCLs are negative for CD45, but these will usually stain for CD3, CD30, or the ALK protein.

Nonhematopoietic neoplasms are also in the differential diagnosis of LL and BL. The cells of these lymphomas are more intermediate in size rather than truly large. For this reason, and because they are common in children, the differential diagnosis includes small round-cell malignancies, such as *neuroblastoma*, *rhabdomyosarcoma*, and *Ewing sarcoma/primitive neuroectodermal tumor (PNET)*. Morphologic features are helpful: The nonhematopoietic neoplasms lack lymphoglandular bodies, and the cells form aggregates in smears. The cells of neuroblastoma form rosettes, have fibrillary neuropil, and show nuclear molding. The cells of rhabdomyosarcoma have a greater degree of anisonucleosis than LL or BL, more abundant, tapered cytoplasm, and are frequently binucleated or multinucleated.¹¹⁵ Immunostains for neural, muscle, and lymphoid markers help to confirm the morphologic impression.

Occasionally, one of the large cell lymphomas resembles *reactive lymphoid hyperplasia*. This is particularly true of a subtype of DLBL, the T-cell/histiocyte rich DLBL.⁵⁸ In this tumor, the neoplastic large cells are outnumbered by reactive T-cells. To identify the monoclonal population by FCM, it is necessary to gate on the large cells using forward scatter. Even so, FCM may give a false-negative result. Some cases of peripheral T-cell lymphoma, those composed of a mixture of small and large cells, also mimic reactive hyperplasia. In most cases, however, there is a sufficient number of atypical small and large cells, with nuclear membrane irregularities (indentations, grooves, knobs), to rule out a reactive hyperplasia.^{28,106} IP often demonstrates aberrant patterns of expression of T-cell antigen, supporting the neoplastic nature of the lesion.

Mononuclear and classical (binucleate) RS cells are frequently seen in ALCL. The sheer number of these pleomorphic cells,⁸² and the paucity of inclusion-like macronucleoli help to exclude HL. In borderline cases, immunostaining for CD15 (positive in RS cells and negative in the hallmark cells of ALCL) or EMA and ALK (negative in classical HL, positive in ALCL) and, if necessary, T-cell receptor rearrangements (detectable by molecular testing in ALCL) can be helpful.

Extensive sclerosis in some cases of DLBL, particularly those in the mediastinum, may yield sparsely cellular smears. The few lymphoid cells present are often crushed, and the remaining fragments of fibrous tissue might be mistaken for a *spindle-cell neoplasm*⁶² or *granulomatous inflammation*. Knowledge of this pitfall (and thus carefully searching for large, atypical lymphoid forms) is usually rewarding.

The large cell type of peripheral T-cell lymphoma is morphologically identical to *DLBL*; the distinction depends on IP. Although it expresses B-cell antigens, *DLBL* is otherwise a heterogeneous group that does not exhibit any one immunophenotype; the most common is CD5–, CD10–, CD23–, but one or more of these markers can be positive, particularly CD10. The distinction between *DLBL*, particularly T-cell or histiocyte rich *DLBL*, and *HL* can be problematic. Again, immunomarkers are useful; in general, RS cells (except in the nodular lymphocyte predominant *HL*) are positive for CD15 and CD30, and most *NHLs* are negative.¹⁶ Demonstrating a t(14;18) rearrangement, seen in 30% of *DLBLs*, can be helpful in excluding *HL*.⁹⁰

ALCLs express a T-cell or null cell phenotype and may or may not express the ALK protein, thus the diagnosis is not restricted to the ALK-positive cases. Rather, a diagnosis of *ALCL* is based on both morphology, immunophenotype, and the exclusion of mimics. Morphologically similar B-cell tumors exist, and some of these may even express CD30, but because the anaplastic B-cell neoplasms have clinical characteristics and behavior similar to other *DLBLs*, they are categorized as *DLBL* and not as *ALCL*.

LL is almost always positive for Tdt, a highly specific and sensitive marker of lymphoblasts that is not present in other lymphomas or reactive lymphocytes in a lymph node. With an aspiration of a mediastinal mass, however, the differential diagnosis includes *thymoma*, particularly type B1 thymoma (lymphocyte-rich thymoma), in which the neoplastic epithelial cells are frequently inconspicuous. Not only are the lymphocytes of a B1 thymoma Tdt positive, like the cells of lymphoblastic lymphoma; the two cell types—B1 thymoma lymphocytes and lymphoblasts—are often morphologically indistinguishable.^{116,117} FCM can be helpful in this distinction, because T-cell (and other antigen) expression patterns are different.¹¹⁷

The diagnosis of *BL* is based on a combination of clinical, morphologic, immunophenotypic, and cytogenetic features. The endemic, sporadic, and HIV-associated forms have characteristic clinical presentations; extranodal sites are involved in the endemic (jaw/orbit) and sporadic (abdomen) forms, and the immunodeficiency-associated forms. *BLs* express B-cell antigens and CD10, and are negative for Tdt. The morphologic features (clumped chromatin, blue cytoplasm with lipid vacuoles, high mitotic rate, apoptosis, tingible-body macrophages) are highly characteristic. The c-myc rearrangement involving chromosome 8 is highly sensitive, but it is not entirely specific because it may occur in *DLBL*. A difficult distinction is between *BL* and *Burkitt-like lymphoma*, a tumor with a proliferative index of nearly 100% but greater pleomorphism in nuclear size and shape, and nucleoli that are fewer in number but more prominent than in *BL*. The diagnosis of *Burkitt-like lymphoma* should be reserved for cases that, like *BL*, show the c-myc translocation.¹⁶ *Burkitt-like lymphoma* is a

poorly reproducible histologic category,⁹⁹ and fortunately, the distinction between it and *BL* is usually not important because most oncologists treat them in a similar fashion.

PTLDs occur only in solid organ or bone marrow transplant recipients. In such patients, the diagnosis of *PTLD* should always be considered. In situ hybridization for EBER is quite helpful; a positive result for EBER in a majority of the lesional cells provides strong support for the diagnosis of *PTLD*. About 20% of *PTLDs* are EBV-negative, however, and a negative result is likely to be inconclusive for a diagnosis of *PTLD*.

Myeloid sarcomas, tumors of immature myeloid cells that occur in bones or extramedullary locations, may mimic *NHL*. IP for myeloid antigens (CD13, CD33, CD117) or histochemistry for myeloid enzymes (myeloperoxidase, chloracetate esterase) is essential for diagnosis. Once a diagnosis of myeloid sarcoma is established, genetic analysis, whether by conventional cytogenetics, FISH, or PCR, is essential for clinical management because the genetic profile stratifies the acute myeloid leukemias and myeloid sarcomas into prognostically relevant subgroups.¹⁶ A repeat FNA should be considered to obtain material for such genetic studies if none was available from the initial specimen.

Finally, the differential diagnosis includes the rare *histiocytic and dendritic cell neoplasms* (Table 11.8), which together represent less than 1% of tumors in lymph nodes.¹⁶ They are diagnosed on the basis of their phenotypic resemblance to their presumed cell of origin. Histiocytic sarcoma can be morphologically indistinguishable from a *DLBL* or an *ALCL*. Unlike the latter two tumors, however, it expresses the histiocyte markers CD68 and CD163. Because myeloid lesions, like myeloid sarcomas, are also positive for CD68, the absence of myeloid markers (e.g., myeloperoxidase) and demonstration of a more mature histologic appearance and immunophenotype (e.g., CD34 negativity) must be established before the diagnosis of histiocytic sarcoma is made.

TABLE 11.8 HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS AND THEIR IDENTIFYING MARKERS

Tumor	Identifying Markers
Histiocytic sarcoma	CD68, CD163
Langerhans cell histiocytosis	S-100, CD1a, Birbeck granules
Langerhans cell sarcoma	S-100, CD1a, Birbeck granules
Interdigitating dendritic cell sarcoma	S-100
Dendritic cell sarcoma, not otherwise specified	S-100, CD1a, no Birbeck granules
Follicular dendritic cell sarcoma	CD21, CD23, CD35

The family of dendritic cells, whose function is antigen presentation to other cells of the immune system, includes the Langerhans cells (in the skin), interdigitating dendritic cells (in the lymph nodes), and follicular dendritic cells (in lymph node follicles). Both Langerhans cells and interdigitating dendritic cells are positive for S-100 protein, but in other ways they are phenotypically distinct. *Langerhans cell histiocytosis* can affect children or adults and be unifocal or multifocal. Common sites are bones, skin, and lungs. The key to diagnosis is recognizing the Langerhans cell, which has a grooved, indented, or lobulated nucleus and abundant cytoplasm, in a background of eosinophils, histiocytes, neutrophils, and lymphocytes. Tumors with overtly malignant cytologic features are *Langerhans cell sarcomas*.¹⁶

Interdigitating dendritic cell sarcoma/tumor is an extremely rare tumor that occurs predominantly in adults. The cells can have an epithelioid or spindle-cell appearance. Like the Langerhans cells, interdigitating dendritic cells are positive for S-100 protein, but they lack CD1a, CD4, and Birbeck granules.

Because *follicular dendritic sarcomas* usually have a spindle-cell appearance, they are discussed later in the chapter.

Nonlymphoid Neoplasms

Carcinomas, melanomas, germ cell tumors, and sarcomas can all metastasize to lymph nodes; carcinomas are the most frequent. Because one usually has a clinical history of a malignancy, and because the cytomorphology of these tumors is so alien to that of the normal lymph node milieu, metastases to lymph nodes are readily recognized as malignant.

Carcinomas

Small cell carcinoma of the lung commonly metastasizes to lymph nodes. Large cell carcinomas of the aerodigestive tract, lung, thyroid, breast, kidney, and colorectal region also metastasize to the lymph nodes, and in some instances the primary tumor is occult. In such instances, smears may contain clues that point to a likely primary site.

CYTOMORPHOLOGY OF LARGE CELL CARCINOMAS:

- cells predominantly in clusters
- no lymphoglandular bodies
- abundant necrosis: favor colorectal, lung, and renal cell carcinoma
- signet ring cells: favor gastric and breast carcinoma
- abundant clear cytoplasm: favor renal cell carcinoma and ovarian carcinoma
- intranuclear inclusions: favor papillary thyroid carcinoma and melanoma

Some aspirates from metastatic squamous cell carcinomas with cystic degeneration contain only necrotic parakeratotic cells and amorphous debris, and thus imitate a branchial cleft cyst or epidermal inclusion cyst. In the absence of convincingly malignant cells, smudgy nuclear hyperchromasia and pyknosis should prompt the diagnosis suspicious for squamous cell carcinoma.

Nasopharyngeal carcinoma (NPC) commonly manifests itself, like lymphoma, as an enlarged cervical lymph node.

CYTOMORPHOLOGY OF NASOPHARYNGEAL CARCINOMA:

- clusters of undifferentiated large cells
- large nuclei with pale chromatin
- with or without prominent nucleolus
- moderate amount of cytoplasm
- lymphocytes, often commingled with epithelial cells
- lymphoglandular bodies

An overwhelming population of lymphocytes can sometimes obscure the clusters of malignant epithelial cells of metastatic NPC, which then resemble dendritic-lymphocytic aggregates¹¹⁸ (Fig. 11.29). The malignant cells are not keratinized, thus the cytoplasm is thin and transparent in Papanicolaou-stained smears. In lymph node samples with few metastatic NPC cells, the rare large malignant cells with their prominent nucleoli can be mistaken for the RS cells of HL. Immunostains can be quite helpful. The cells of NPC are cytokeratin-positive and CD45-negative. Virtually all nasopharyngeal carcinomas are positive for EBV DNA or RNA (see Fig. 11.29 inset).

Malignant Melanoma

Malignant melanoma is aptly named *the great masquerader*. Lymph nodes are among the most commonly aspirated metastatic sites.¹¹⁹

CYTOMORPHOLOGY OF MELANOMA:

- dispersed single cells and loose clusters
- epithelioid, spindle, or pleomorphic shapes
- nuclei eccentrically placed, commonly binucleated
- nuclear inclusions
- single small to large nucleoli
- cytoplasm: melanin pigment variable, vacuoles variable
- no lymphoglandular bodies

Melanin pigment is seen in less than 50% of aspirate smears.¹¹⁹ The differential diagnosis of amelanotic malignant melanoma includes DLBL because both show a

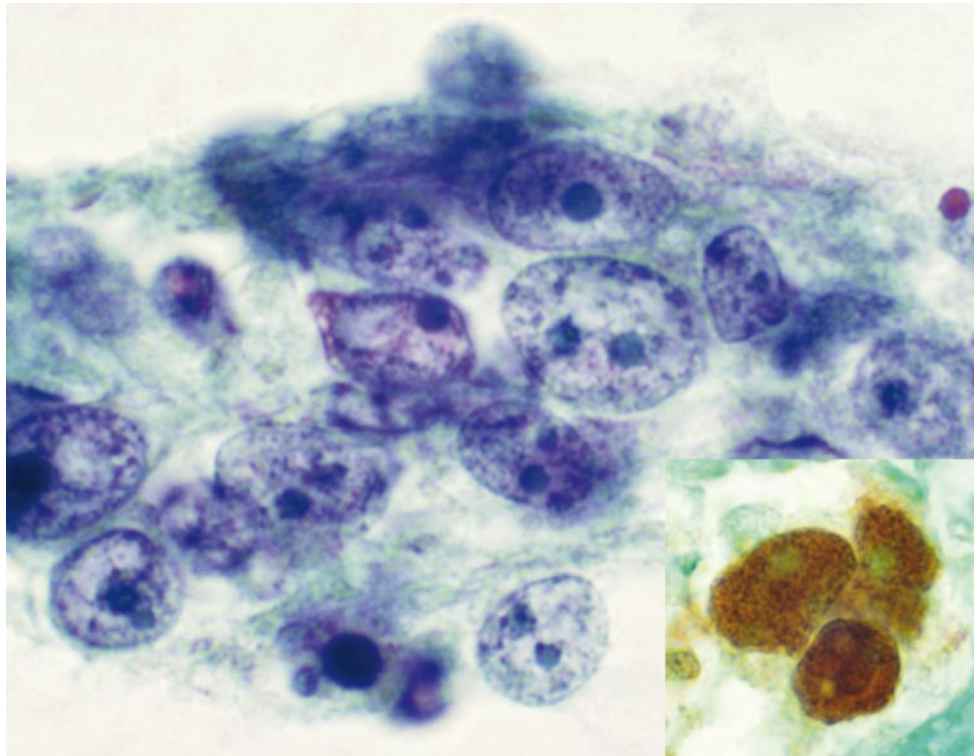


Figure 11.29 Nasopharyngeal (undifferentiated) carcinoma. A loose but definite cluster of epithelial cells is almost obscured by the large number of small round lymphocytes (Papanicolaou stain). *Inset:* The malignant cells show nuclear staining for Epstein-Barr virus encoded RNAs (EBER) by in situ hybridization.

single cell pattern of monomorphic large cells. The eccentric nuclear placement (plasmacytoid nuclei), lack of lymphoglandular bodies, and frequent binucleation are clues to the diagnosis of malignant melanoma (Fig. 11.30).

Seminoma or Germinoma

Testicular and mediastinal seminomas or germinomas commonly metastasize to deep lymph nodes of the abdomen and chest, respectively.

CYTOMORPHOLOGY OF SEMINOMA AND GERMINOMA:

- dispersed large cells
- macronucleolus
- voluminous cytoplasm, peripherally placed vacuoles with large blister-like quality
- small round lymphocytes and lymphoglandular bodies
- granulomas
- “tigroid” background

Much of the literature regarding seminoma has focused on the smear background, in which strands of cytoplasm and proteinaceous fluid are arranged in a reticular or linear network termed the “*tigroid pattern*.” This background is not universally present, nor is it entirely specific for seminoma; it is also seen with clear cell carcinomas. Lymphocytes are common in

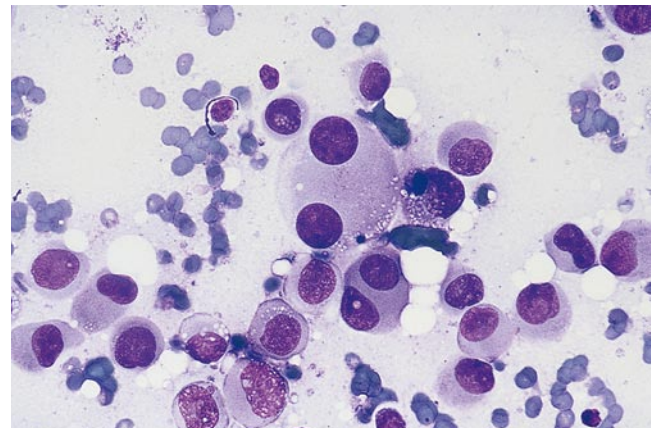


Figure 11.30 Malignant melanoma. Large nonpigmented cells are distributed in a single cell pattern. Binucleated cells with wide spacing between the mirror-image nuclei are a strong clue to the diagnosis (Romanowsky stain).

seminoma, and thus lymphoglandular bodies may be present, so that confusion with a malignant lymphoma is possible. Foci of cell clustering and the marked vacuolization of germ cells are helpful in this regard. FNA smears of other malignant germ cell tumors, yolk sac carcinoma, and embryonal carcinoma mimic those of non-small cell carcinoma. The tigroid pattern is not evident, nor is a background population of lymphocytes present. Sometimes, one finds hyaline globules in aspirates of yolk sac carcinoma where they appear as spherical glassy structures.

Sarcomas

Most sarcomas tend not to metastasize to lymph nodes: less than 3% of patients with sarcoma develop lymph node metastases.¹²⁰ A subset of sarcomas breaks rank with the “anti-lymph node” imperative, however.

Sarcomas that more commonly involve lymph nodes include:

- synovial sarcoma
- epithelioid sarcoma
- angiosarcoma
- rhabdomyosarcoma
- Kaposi sarcoma
- follicular dendritic cell sarcoma

As one of the more common sarcomas to metastasize to lymph nodes, *synovial sarcoma* has monomorphic, bland-appearing ovoid nuclei with finely granular chromatin, smooth nuclear outlines, and scant amounts of tapering cytoplasm. Rarely, acinar structures reflecting the glandular epithelial component are found.

Lymph node involvement by *Kaposi sarcoma* occurs in an endemic form, and sporadically in immunodeficient states such as renal transplantation and AIDS. Smears are variably cellular with abundant red cells, and the cytomorphology is similar to that of any spindle cell sarcoma.¹²¹ Hyaline globules, a typical histologic feature, are usually difficult to find in smears. Definitive diagnosis

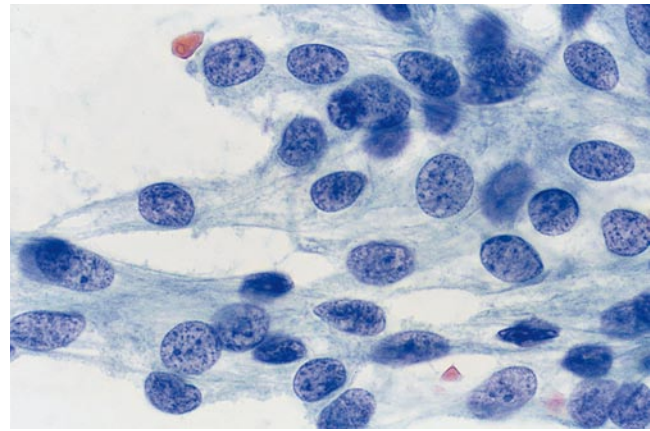


Figure 11.31 Follicular dendritic cell sarcoma. Delicate cytoplasmic processes extend from these clustered cells with smooth, spindle-shaped nuclei (Romanowsky stain).

nearly always requires immunocytochemistry. The spindle cells are positive for CD31 and CD34.

Follicular dendritic cell sarcoma is a rare tumor of young to middle-aged adults that arises in lymph nodes and in extranodal sites. It is a slow-growing tumor with metastatic potential. Smears show loose, flat aggregates and single cells with oval and spindle shapes.¹²² Intermediate-sized nuclei have smooth borders and small, inapparent nucleoli (Fig. 11.31). They are positive for one or more of the follicular dendritic cell markers CD21, CD23, and CD35, and are negative for CD45.

REFERENCES

1. Nason RW, Abdulrauf BM, Stranc MF: The anatomy of the accessory nerve and cervical lymph node biopsy. *Am J Surg* 2000;180(3):241-243.
2. Landgren O, Porwit MacDonald A, et al.: A prospective comparison of fine-needle aspiration cytology and histopathology in the diagnosis and classification of lymphomas. *Hematol J* 2004;5(1):69-76.
3. Young NA, Al-Saleem T: Diagnosis of lymphoma by fine-needle aspiration cytology using the revised European-American classification of lymphoid neoplasms. *Cancer* 1999;87:325-345.
4. Glant MD: Cytopathology of lymph nodes in nonspecific reactive hyperplasia: Prognostication and differential diagnosis. *Am J Clin Pathol* 1997;108:S31-S55.
5. Tsang WYW, Chan JKC: Spectrum of morphologic changes attributable to fine-needle aspiration. *Hum Pathol* 1992;23:562-565.
6. Behm FG, O'Dowd GJ, Frable WJ: Fine needle aspiration effects on benign lymph node. *Am J Clin Pathol* 1985;82:195-198.
7. Lioe TF, Elliott H, Allen DC, Spence RAJ: The role of fine needle aspiration cytology (FNAC) in the investigation of superficial lymphadenopathy; uses and limitations of the technique. *Cytopathology* 1999;10:291-297.
8. Nasuti JF, Yu G, Boudousquie A, Gupta P: Diagnostic value of lymph node fine needle aspiration cytology: An institutional experience of 387 cases observed over a 5-year period. *Cytopathology* 2000;11:18-31.
9. Prasad RR, Narasimhan R, Sankaran V, Veliath AJ: Fine-needle aspiration cytology in the diagnosis of superficial lymphadenopathy: an analysis of 2,418 cases. *Diagn Cytopathol* 1996;15:382-386.
10. Steel BL, Schwartz MR, Ramzy I: Fine needle aspiration biopsy in the diagnosis of lymphadenopathy in 1103 patients. Role, limitations and analysis of diagnostic pitfalls. *Acta Cytol* 1995;39:76-81.
11. Lee RE, Kalaitis J, Kalis O, et al.: Lymph node examination by fine needle aspiration in patients with known or suspected malignancy. *Acta Cytol* 1987;31:563-572.
12. Dong HY, Harris NL, Preffer FI, Pitman MB: Fine-needle aspiration biopsy in the diagnosis and classification of primary and recurrent lymphoma: a retrospective analysis of the utility of cytomorphology and flow cytometry. *Mod Pathol* 2001;14:472-481.
13. Kardos TF, Vinson JH, Behm FG, et al.: Hodgkin's disease: diagnosis by fine needle aspiration biopsy. Analysis of cytologic criteria from a selected series. *Am J Clin Pathol* 1986;86:286-291.
14. Fulciniti F, Vetrani A, Zeppa P, et al.: Hodgkin's disease: diagnostic accuracy of fine needle aspiration; A report based on 62 consecutive cases. *Cytopathology* 1994;5:226-233.
15. Hehn ST, Grogan TM, Miller TP: Utility of fine-needle aspiration as a diagnostic technique in lymphoma. *J Clin Oncol* 2004;22(15):3046-3052.
16. Jaffe ES, Harris NL, Stein M, Vardiman JW (eds): World Health Organization Classification of Tumours. Pathology and

- Genetics of Tumours and Haematopoietic and Lymphoid Tissues. Lyon, France, IARC Press, 2001.
17. Wakely PE Jr: Fine needle aspiration cytopathology in diagnosis and classification of malignant lymphoma: accurate and reliable? *Diagn Cytopathol* 2000;22:120-125.
 18. Meda BA, Buss DH, Woodruff RD, et al.: Diagnosis and subclassification of primary and recurrent lymphomas. The usefulness and limitations of combined fine needle aspiration cytomorphology and flow cytometry. *Am J Clin Pathol* 2000;113:688-699.
 19. Nicol TL, Silberman M, Rosenthal DL, Borowitz MJ: The accuracy of combined cytopathology and flow cytometric analysis of fine-needle aspirates of lymph nodes. *Am J Clin Pathol* 2000;114:18-28.
 20. Young NA, Al-Saleem TI, Ehya H, Smith MR: Utilization of fine-needle aspiration cytology and flow cytometry in the diagnosis and subclassification of primary and recurrent lymphoma. *Cancer* 1998;84:252-261.
 21. Jacobs JC, Katz RL, Shabb N, et al.: Fine needle aspiration of lymphoblastic lymphoma. A multiparameter diagnostic approach. *Acta Cytol* 1992;36:887-894.
 22. Wakely PE Jr, Kornstein MJ: Aspiration cytopathology of lymphoblastic lymphoma and leukemia: the MCV experience. *Pediatr Pathol Lab Med* 1996;16:243-252.
 23. Kussick SJ, Kalnoski M, Brazier RM, Wood BL: Prominent clonal B-cell populations identified by flow cytometry in histologically reactive lymphoid proliferations. *Am J Clin Pathol* 2004;121(4):464-472.
 24. Simsir A, Fetsch P, Stetler-Stevenson M, Abati A: Immunophenotypic analysis of non-Hodgkin lymphomas in cytologic specimens: A correlative study of immunocytochemical and flow cytometric techniques. *Diagn Cytopathol* 1999;20:278-284.
 25. Tani E, Ersoz C, Svedmyr E, et al.: Fine needle aspiration cytology and immunocytochemistry of Hodgkin's disease—suppurative type. *Diagn Cytopathol* 1998;18:437-440.
 26. Chhieng DC, Cangiarella JE, Symmans WF, Cohen JM: Fine-needle aspiration cytology of Hodgkin disease. A study of 89 cases with emphasis on the false-negative cases. *Cancer* 2001;93:52-59.
 27. Dunphy CH, Ramos R: Combining fine-needle aspiration and flow cytometric immunophenotyping in evaluation of nodal and extranodal sites for possible lymphoma: A retrospective review. *Diagn Cytopathol* 1997;16:200-206.
 28. Yao JL, Cangiarella JE, Cohen JM, Chhieng DC: Fine-needle aspiration biopsy of peripheral T-cell lymphomas. A cytologic and immunophenotypic study of 33 cases. *Cancer* 2001;93:151-159.
 29. Braylan RC, Orfao A, Borowitz MJ, Davis BH: Optimal number of reagents required to evaluate hematolymphoid neoplasias: Results of an international consensus meeting. *Cytometry* 2001;46:23-27.
 30. Zeppa P, Marino G, Troncone G, et al.: Fine-needle cytology and flow cytometry immunophenotyping and subclassification of non-Hodgkin lymphoma: A critical review of 307 cases with technical suggestions. *Cancer* 2004;102(1):55-65.
 31. Mathiot C, Decaudin D, Klijanienko J, et al.: Fine-needle aspiration cytology combined with flow cytometry immunophenotyping is a rapid and accurate approach for the evaluation of suspicious superficial lymphoid lesions. *Diagn Cytopathol* 2006;34(7):472-478.
 32. Al Shanqeety O, Mourad WA: Diagnosis of peripheral T-cell lymphoma by fine-needle aspiration biopsy: A cytomorphologic and immunophenotypic approach. *Diagn Cytopathol* 2000;23:375-379.
 33. Grosso LE, Collins BT: DNA polymerase chain reaction using fine needle aspiration biopsy smears to evaluate non-Hodgkin's lymphoma. *Acta Cytol* 1999;43:837-841.
 34. Hughes JH, Katz RL, Fonseca GA, Cabanillas FF: Fine-needle aspiration cytology of mediastinal non-Hodgkin's nonlymphoblastic lymphoma. *Cancer* 1998;84:26-35.
 35. Jeffers MD, McCorriston J, Farquharson MA, et al.: Analysis of clonality in cytologic material using the polymerase chain reaction (PCR). *Cytopathology* 1997;8:114-121.
 36. Vianello F, Tison T, Radossi P, et al.: Detection of B-cell monoclonality in fine needle aspiration by PCR analysis. *Leuk Lymphoma* 1998;29:179-185.
 37. Galindo LM, Garcia FU, Hanau CA, et al.: Fine-needle aspiration biopsy in the evaluation of lymphadenopathy associated with cutaneous T-cell lymphoma (mycosis fungoides/Sézary syndrome). *Am J Clin Pathol* 2000;113:865-871.
 38. Katz RL, Caraway NP, Gu J, et al.: Detection of chromosome 11q13 breakpoints by interphase fluorescence in situ hybridization. A useful ancillary method for the diagnosis of mantle cell lymphoma. *Am J Clin Pathol* 2000;114:248-257.
 39. Safley AM, Buckley PJ, Creager AJ, et al.: The value of fluorescence in situ hybridization and polymerase chain reaction in the diagnosis of B-cell non-Hodgkin lymphoma by fine-needle aspiration. *Arch Pathol Lab Med* 2004;128(12):1395-1403.
 40. Morrison C, Palatini J, Riggensbach J, et al.: Fine-needle aspiration biopsy of non-Hodgkin lymphoma for use in expression microarray analysis. *Cancer* 2006;108(5):311-318.
 41. Goy A, Stewart J, Barkoh BA, et al.: The feasibility of gene expression profiling generated in fine-needle aspiration specimens from patients with follicular lymphoma and diffuse large B-cell lymphoma. *Cancer* 2006;108(1):10-20.
 42. O'Dowd GJ, Frable WJ, Behm FG: Fine needle aspiration cytology of benign lymph node hyperplasia. Diagnostic significance of lymphohistiocytic aggregates. *Acta Cytol* 1985;29:554-558.
 43. Suh YK, Shabaik A, Meurer WT, Shin SS: Lymphoid cell aggregates: A useful clue in the fine-needle aspiration diagnosis of follicular lymphomas. *Diagn Cytopathol* 1997;17:467-471.
 44. Söderström N: The free cytoplasmic fragments of lymphoglandular tissue (lymphoglandular bodies). A preliminary presentation. *Scand J Haematol* 1968;5:138-152.
 45. Flanders E, Kornstein MJ, Wakely PE Jr, et al.: Lymphoglandular bodies in fine needle aspiration cytology. *Am J Clin Pathol* 1993;99:566-569.
 46. Stani J: Cytologic diagnoses of reactive lymphadenopathy in fine needle aspiration biopsy specimens. *Acta Cytol* 1987;31:8-13.
 47. Hecht JL, Cibas ES, Kutok JL: Fine-needle aspiration cytology of lymphoproliferative disorders in the immunosuppressed patient: The diagnostic utility of in situ hybridization for Epstein-Barr virus. *Diagn Cytopathol* 2002;26(6):360-365.
 48. Meyer L, Gibbons D, Ashfaq R, et al.: Fine-needle aspiration findings in Castleman's disease. *Diagn Cytopathol* 1999;21:57-60.
 49. Taylor GB, Smeeton WMI: Cytologic demonstration of "dysplastic" follicular dendritic cells in a case of hyaline-vascular Castleman's disease. *Diagn Cytopathol* 2000;22:230-234.
 50. Gupta RK: Fine needle aspiration cytodiagnosis of toxoplasmic lymphadenitis. *Acta Cytol* 1997;41:1031-1034.
 51. Zaharopoulos P: Demonstration of parasites in toxoplasma lymphadenitis by fine-needle aspiration cytology: Report of two cases. *Diagn Cytopathol* 2000;22:11-15.
 52. Ellison E, Lapuerta P, Martin SE: FNA in HIV+ patients: results from a series of 655 patients. *Cytopathology* 1998;9:222-229.
 53. Reid AJ, Miller RF, Kocjan GI: Diagnostic utility of fine needle aspiration (FNA) cytology in HIV-infected patients with lymphadenopathy. *Cytopathology* 1998;9:230-239.
 54. Martin-Bates E, Tanner A, Suvana SK, et al.: Use of fine needle aspiration cytology for investigating lymphadenopathy in HIV positive patients. *J Clin Pathol* 1993;46:546-566.

55. Jayaram G, Chew MT: Fine needle aspiration cytology of lymph nodes in HIV-infected patients. *Acta Cytol* 2000;44:960-966.
56. Rahemtullah A, Reichard KK, Preffer FI, et al.: A double-positive CD4+CD8+ T-cell population is commonly found in nodular lymphocyte predominant Hodgkin lymphoma. *Am J Clin Pathol* 2006;126(5):805-814.
57. Iyer VK, Kapila K, Verma K: Fine needle aspiration cytology of dermatopathic lymphadenitis. *Acta Cytol* 1998;42:1347-1351.
58. Galindo LM, Havlioglu N, Grosso LE: Cytologic findings in a case of T-cell rich B-cell lymphoma: Potential diagnostic pitfall in FNA of lymph nodes. *Diagn Cytopathol* 1996;14:253-257.
59. Frable MA, Frable WJ: Fine needle aspiration biopsy in the diagnosis of sarcoid of the head and neck. *Acta Cytol* 1984;28:175-177.
60. Perez-Guillermo M, Perez JS, Espinosa Parra FJ: Asteroid bodies and calcium oxalate crystals: Two infrequent findings in fine-needle aspirates of parotid sarcoidosis. *Diagn Cytopathol* 1992;8:248-252.
61. Khurana KK, Stanley MW, Powers CN, Pitman MB: Aspiration cytology of malignant neoplasms associated with granulomas and granuloma-like features: diagnostic dilemmas. *Cancer* 1998;84:84-91.
62. Silverman JF, Raab SS, Park HK: Fine-needle aspiration cytology of primary large cell lymphoma of the mediastinum: Cytomorphologic findings with potential pitfalls in diagnosis. *Diagn Cytopathol* 1993;9:209-215.
63. Stastny JF, Wakely PE Jr, Frable WJ: Cytologic features of necrotizing granulomatous inflammation consistent with cat scratch disease. *Diagn Cytopathol* 1996;15:108-115.
64. Donnelly A, Hendricks G, Martens S, et al.: Cytologic diagnosis of cat scratch disease (CSD) by fine-needle aspiration. *Diagn Cytopathol* 1995;13:103-106.
65. Ellison E, Lapuerta P, Martin SE: Fine needle aspiration diagnosis of mycobacterial lymphadenitis. Sensitivity and predictive value in the United States. *Acta Cytol* 1999;43:153-157.
66. Das DK, Bhambhani S, Pant JN, et al.: Superficial and deep-seated tuberculous lesions: Fine-needle aspiration cytology diagnosis of 574 cases. *Diagn Cytopathol* 1992;8:211-215.
67. Maygarden SJ, Flanders E: Mycobacteria can be seen as "negative images" in cytology smears from patients with acquired immunodeficiency syndrome. *Mod Pathol* 1989;2:239-243.
68. Deshpande AH, Nayak S, Munshi MM: Cytology of sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). *Diagn Cytopathol* 2000;22:181-185.
69. Trautman BC, Stanley MW, Goding GS, Rosai J: Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease): Diagnosis by fine needle aspiration. *Diagn Cytopathol* 1991;7:513-516.
70. Tsang WYW, Chan JKC: Fine-needle aspiration cytologic diagnosis of Kikuchi's lymphadenitis. A report of 27 cases. *Am J Clin Pathol* 1994;102:454-458.
71. Viguer JM, Jimenez-Heffernan JA, Perez P, et al.: Fine-needle aspiration cytology of Kikuchi's lymphadenitis: a report of ten cases. *Diagn Cytopathol* 2001;25:220-224.
72. Pai MR, Adhikari P, Coimbatore RV, Ahmed S: Fine needle aspiration cytology in systemic lupus erythematosus lymphadenopathy. A case report. *Acta Cytol* 2000;44(1):67-69.
73. Kardos TF, Kornstein MJ, Frable WJ: Cytology and immunocytology of infectious mononucleosis in fine needle aspirates of lymph nodes. *Acta Cytol* 1988;32:722-726.
74. Stanley MW, Steeper TA, Horwitz CA, et al.: Fine needle aspiration of lymph nodes in patients with acute infectious mononucleosis. *Diagn Cytopathol* 1990;6:323-329.
75. Gascoyne RD: Establishing the diagnosis of lymphoma: From initial biopsy to clinical staging. *Oncology* 1998;12(10 Suppl 8):11-16.
76. Rassidakis GZ, Tani E, Svedmyr E, et al.: Diagnosis and subclassification of follicle center and mantle cell lymphomas on fine-needle aspirates. A cytology and immunocytochemical approach based on the revised European-American lymphoma (REAL) classification. *Cancer* 1999;87:216-223.
77. Wakely PE Jr: Aspiration cytopathology of malignant lymphoma. Coming of age. *Cancer* 1999;87:322-324.
78. Caraway NP: Strategies to diagnose lymphoproliferative disorders by fine-needle aspiration by using ancillary studies. *Cancer* 2005;105(6):432-442.
79. Zhang JR, Raza AS, Greaves TS, Cobb CJ: Fine-needle aspiration diagnosis of Hodgkin lymphoma using current WHO classification—re-evaluation of cases from 1999-2004 with new proposals. *Diagn Cytopathol* 2006;34(6):397-402.
80. Chhieng DC, Cohen JM, Cangiarella JF: Cytology and immunophenotyping of low-and intermediate-grade B-cell non-Hodgkin's lymphomas with a predominant small-cell component: A study of 56 cases. *Diagn Cytopathol* 2001;24:90-97.
81. Zeng G, Ewton A, Zu Y, et al.: Quantitative analysis of CD4/CD8 ratios by flow cytometry as an adjunct in diagnosis of Hodgkin's lymphoma [abstract]. *Mod Pathol* 2005;18(suppl):259A-260A.
82. Bizjak-Schwarzbartl M: Large cell anaplastic Ki-1+ non-Hodgkin's lymphoma vs. Hodgkin's disease in fine needle aspiration biopsy samples. *Acta Cytol* 1997;41:351-356.
83. Tani EM, Christensen B, Porwit A, Skoog L: Immunocytochemical analysis and cytomorphologic diagnosis on fine-needle aspirates of lymphoproliferative disease. *Acta Cytol* 1988;32:209-215.
84. Shin HJ, Caraway NP, Katz RL: Cytomorphologic spectrum of small lymphocytic lymphoma in patients with an accelerated clinical course. *Cancer* 2003;99(5):293-300.
85. Bentz JS, Rowe LR, Anderson SR, et al.: Rapid detection of the t(11;14) translocation in mantle cell lymphoma by interphase fluorescence in situ hybridization on archival cytopathologic material. *Cancer* 2004;102(2):124-131.
86. Caraway NP, Gu J, Lin P, et al.: The utility of interphase fluorescence in situ hybridization for the detection of the translocation t(11;14)(q13;q32) in the diagnosis of mantle cell lymphoma on fine-needle aspiration specimens. *Cancer* 2005;105(2):110-118.
87. Wojcik EM, Katz RL, Fanning TV, et al.: Diagnosis of mantle cell lymphoma on tissue acquired by fine needle aspiration in conjunction with immunocytochemistry and cytokinetic studies: possibilities and limitations. *Acta Cytol* 1995;39:909-915.
88. Hughes JH, Caraway NP, Katz RL: Blastic variant of mantle-cell lymphoma: cytomorphologic, immunocytochemical, and molecular genetic features of tissue obtained by fine-needle aspiration biopsy. *Diagn Cytopathol* 1998;19:59-62.
89. Richmond J, Bryant R, Trotman W, et al.: FISH detection of t(14;18) in follicular lymphoma on Papanicolaou-stained archival cytology slides. *Cancer* 2006;108(3):198-204.
90. Gong Y, Caraway N, Gu J, et al.: Evaluation of interphase fluorescence in situ hybridization for the t(14;18)(q32;q21) translocation in the diagnosis of follicular lymphoma on fine-needle aspirates: a comparison with flow cytometry immunophenotyping. *Cancer* 2003;99(6):385-393.
91. Young NA, Al-Saleem TI, Al-Saleem Z, et al.: The value of transformed lymphocyte count in subclassification of non Hodgkin's lymphoma by fine needle aspiration. *Am J Clin Pathol* 1997;108:143-151.
92. Sun W, Caraway NP, Zhang HZ, et al.: Grading follicular lymphoma on fine needle aspiration specimens. Comparison with proliferative index by DNA image analysis and Ki-67 labeling index. *Acta Cytol* 2004;48(2):119-126.

93. Brandao GD, Rose R, McKenzie S, et al.: Grading follicular lymphomas in fine-needle aspiration biopsies: The role of Thin Prep slides and flow cytometry. *Cancer* 2006;108(5):319-323.
94. Matsushima AY, Hamele-Bena D, Osborne BM: Fine-needle aspiration biopsy findings in marginal zone B cell lymphoma. *Diagn Cytopathol* 1999;20:190-198.
95. Wakely PE Jr: Fine needle aspiration cytopathology of malignant lymphoma. In Stanley MW (ed), *Clinics in Laboratory Medicine, Fine Needle Aspiration*. Philadelphia, WB Saunders, 1998, pp. 541-459.
96. Renshaw AA, Voytek TM, Haja J, Wilbur DC: Distinguishing small cell carcinoma from non-small cell carcinoma of the lung: Correlating cytologic features and performance in the College of American Pathologists Non-Gynecologic Cytology Program. *Arch Pathol Lab Med* 2005;129(5):619-623.
97. Sandlund JT, Downing JR, Crist WM: Non-Hodgkin's lymphoma in children. *New Engl J Med* 1996;334:1238-1248.
98. Silverman JF: Fine needle aspiration cytology of cat-scratch disease. *Acta Cytol* 1985;29:542-547.
99. Harris N, Jaffe ES, Diebold J, et al.: The World Health Organization classification of hematologic malignancies report of the clinical advisory committee meeting, Airlie House, Virginia, November, 1997. *Mod Pathol* 2000;13:193-207.
100. Alizadeh AA, Eisen MB, Davis RE, et al.: Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;403(6769):503-511.
101. Rosenwald A, Wright G, Chan WC, et al.: The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002;346(25):1937-1947.
102. Jorgensen JL: State of the Art Symposium: flow cytometry in the diagnosis of lymphoproliferative disorders by fine-needle aspiration. *Cancer* 2005;105(6):443-451.
103. Stastny JE, Almeida MM, Wakely PE Jr, et al.: Fine needle aspiration biopsy and imprint cytology of small non-cleaved cell (Burkitt's) lymphoma. *Diagn Cytopathol* 1995;12:201-207.
104. Hecht JL, Aster JC: Molecular biology of Burkitt's lymphoma. *J Clin Oncol* 2000;18:3707-3721.
105. Troxell ML, Bangs CD, Cherry AM, et al.: Cytologic diagnosis of Burkitt lymphoma. *Cancer* 2005;105(5):310-318.
106. Katz RL, Gritsman A, Cabanillas R, et al.: Fine-needle aspiration cytology of peripheral T-cell lymphoma: A cytologic, immunologic, and cytometric study. *Am J Clin Pathol* 1989;91:120-131.
107. Kinney MC, Kadin ME: The pathologic and clinical spectrum of anaplastic large cell lymphoma and correlation with ALK gene dysregulation. *Am J Clin Pathol* 1999;111 (Suppl.1):S56-S67.
108. Mourad WA, al Nazer M, Tulbah A: Cytomorphologic differentiation of Hodgkin's lymphoma and Ki-1+ anaplastic large cell lymphoma in fine needle aspirates. *Acta Cytol* 2003;47(5):744-748.
109. Ng WK, Ip P, Choy C, Collins RJ: Cytologic and immunocytochemical findings of anaplastic large cell lymphoma: Analysis of ten fine-needle aspiration specimens over a 9-year period. *Cancer* 2003;99(1):33-43.
110. Zakowski MF, Feiner H, Finfer M, et al.: Cytology of extranodal Ki-1 anaplastic large cell lymphoma. *Diagn Cytopathol* 1996;14:155-161.
111. Sgrignoli A, Abati A: Cytologic diagnosis of anaplastic large cell lymphoma. *Acta Cytol* 1997;41:1048-1052.
112. Shin HJ, Thorson P, Gu J, Katz RL: Detection of a subset of CD30+ anaplastic large cell lymphoma by interphase fluorescence in situ hybridization. *Diagn Cytopathol* 2003;29(2):61-66.
113. Kardos TE, Sprague RI, Wakely PE Jr, Frable WJ: Fine needle aspiration biopsy of lymphoblastic lymphoma and leukemia. A clinical, cytologic, and immunologic study. *Cancer* 1987;60:2448-2453.
114. Dusenbery D, Jones DB, Sapp KW, et al.: Cytologic findings in the sarcomatoid variant of large cell anaplastic (Ki-1) lymphoma. A case report. *Acta Cytol* 1993;37:508-514.
115. de Almeida M, Stastny JE, Wakely PE Jr, Frable WJ: Fine-needle aspiration biopsy of childhood rhabdomyosarcoma: Reevaluation of the cytologic criteria for diagnosis. *Diagn Cytopathol* 1994;11:231-236.
116. Friedman HD, Hutchison RE, Kohman LJ, Powers CN: Thymoma mimicking lymphoblastic lymphoma: A pitfall in fine-needle aspiration biopsy interpretation. *Diagn Cytopathol* 1996;14(2):165-169; discussion 169-171.
117. Li S, Juco J, Mann KP, Holden JT: Flow cytometry in the differential diagnosis of lymphocyte-rich thymoma from precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma. *Am J Clin Pathol* 2004;121(2):268-274.
118. Chan MKM, McGuire LJ, Lee JCK: Fine needle aspiration diagnosis of nasopharyngeal carcinoma in cervical lymph nodes. A study of 40 cases. *Acta Cytol* 1989;33:344-350.
119. Perry MD, Gore M, Seigler HE, Johnston WW: Fine needle aspiration biopsy of metastatic melanoma. A morphologic analysis of 174 cases. *Acta Cytol* 1986;30:385-396.
120. Fong Y, Coit DG, Woodruff JM, Brennan MF: Lymph node metastases from soft tissue sarcoma in adults: analysis of data from a prospective database of 1772 sarcoma patients. *Ann Surg* 1993;217:72-77.
121. Hales M, Bottles K, Miller T, et al.: Diagnosis of Kaposi's sarcoma by fine-needle aspiration biopsy. *Am J Clin Pathol* 1987;88:20-25.
122. Wright CA, Nayler SJ, Leiman G: Cytopathology of follicular dendritic cell tumours. *Diagn Cytopathol* 1997;17:138-142.

Liver

BARBARA S. DUCATMAN

NORMAL LIVER

INFECTIONS

Hepatic Abscess
Echinococcal Cyst (Hydatid Cyst)
Other Infections

BENIGN LESIONS

Solitary Cysts
Cirrhosis
Focal Nodular Hyperplasia
Liver Cell Adenoma

Bile Duct Hamartoma and Adenoma
Hemangioma
Angiomyolipoma

MALIGNANT TUMORS

Hepatocellular Carcinoma
Cholangiocarcinoma (Bile Duct Carcinoma)
Hepatoblastoma
Angiosarcoma
Epithelioid Hemangioendothelioma
Metastatic Tumors

Fine-needle aspiration (FNA) is used mainly for the diagnosis of focal lesions of the liver, whereas blind or image-guided percutaneous biopsy with a large-core needle is preferred for diagnosing diffuse liver diseases like hepatitis and cirrhosis, for which large-scale architectural details are important. FNA is usually performed percutaneously under the guidance of computed tomography (CT), magnetic resonance imaging (MRI), or ultrasonography either percutaneously or via endoscopy, and its value is in the diagnosis of malignancies, even those that are small (3 cm or less); sensitivity ranges from 71% to 94% and specificity from 87% to 100%, with an accuracy of 90% to 94%.¹⁻²⁰ False-positive diagnoses are quite uncommon; hepatic dysplasia, bile duct hamartoma, and focal nodular hyperplasia (FNH) have been misdiagnosed as malignant.^{7,21,22} FNA cannot differentiate hepatic adenoma, FNH, and regenerative nodules in cirrhosis. Nevertheless, a benign result is useful because it helps to exclude a malignant neoplasm.

Obtaining brushings during endoscopic retrograde cholangiopancreatography (ERCP) is the preferred technique for diagnosing tumors at the hilum of the liver.²³ When brushings are negative or inconclusive, endosonography-guided FNA can be used to obtain diagnostic cytologic material.^{16-18,23,24}

FNA has also been used in the routine monitoring of liver transplants for acute cellular rejection.²⁵⁻³¹ Slides are air-dried and stained with a Romanowsky stain. Lymphocytes are examined for evidence of activation (enlargement, “blast” forms), and hepatocytes for signs of injury (swelling, vacuolization, necrosis) and cholestasis.³⁰ The technique is not useful for diagnosing causes of chronic rejection.²⁷

Complications of liver FNA are rare and include hemorrhage,^{18,32} pain,¹⁸ bile peritonitis, and anaphylactic shock (after aspiration of an echinococcal cyst). FNA is associated with a low risk (0.1% to 0.6%) of tumor seeding³³⁻³⁷ and recurrence of hepatocellular carcinoma (HCC) after liver transplantation.³⁸ FNA-related mortality has been reported but is quite uncommon (0.6%).^{18,39-42}

NORMAL LIVER

Benign liver cells are commonly seen in aspirates of the liver, and even, occasionally, in aspirates of adjacent organs like the pancreas, right kidney, right adrenal gland, and the right lower lobe of the lung, when the liver is penetrated en route to the target organ. The components of a normal liver aspirate commonly include hepatocytes (Figs. 12.1, 12.2), bile duct epithelium, Kupffer cells, and sheets of mesothelial cells.

CYTOMORPHOLOGY OF HEPATOCYTES:

- large polygonal cells
- isolated cells, thin ribbons (trabeculae), or tissue fragments
- centrally placed, round to oval, and variably sized nucleus
- commonly binucleated
- prominent nucleolus
- intranuclear pseudoinclusions
- abundant granular cytoplasm
- pigment

- lipofuscin (common: a normal pigment related to cellular aging): golden with the Papanicolaou stain and green-brown with a Romanowsky-type stain
- hemosiderin (less common: when present in large quantities it suggests a disorder of iron metabolism): dark brown with the Papanicolaou stain and blue with a Romanowsky-type stain
- bile (seen in cholestasis): dark green with both Papanicolaou and Romanowsky stains (see Fig. 12.2)

CYTOMORPHOLOGY OF BILE DUCT EPITHELIUM:

- cohesive flat sheets
- cuboidal cells smaller than hepatocytes
- evenly spaced nuclei (“honeycomb appearance”)

CYTOMORPHOLOGY OF KUPFFER CELLS:

- resemble macrophages
- vacuolated cytoplasm and may contain pigment, most commonly hemosiderin

DIFFERENTIAL DIAGNOSIS OF NORMAL LIVER CELLS:

- hepatic adenoma
- FNH
- cirrhosis with a regenerative nodule
- nodular transformation of the liver
- steatosis

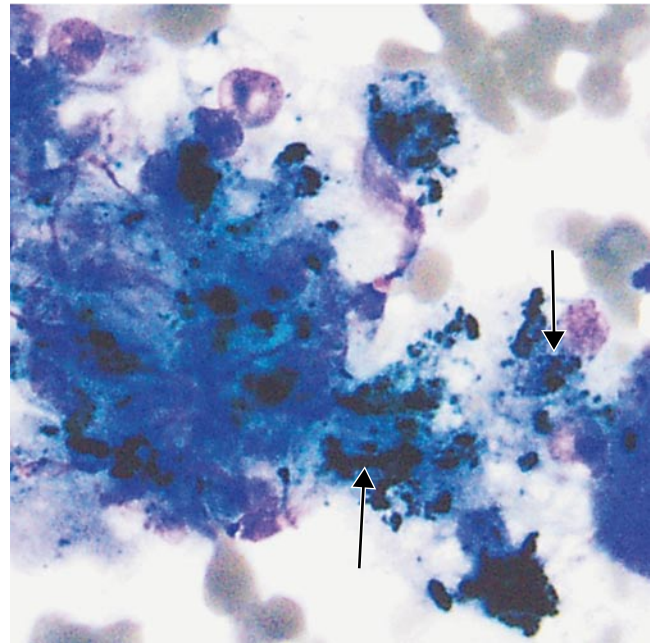
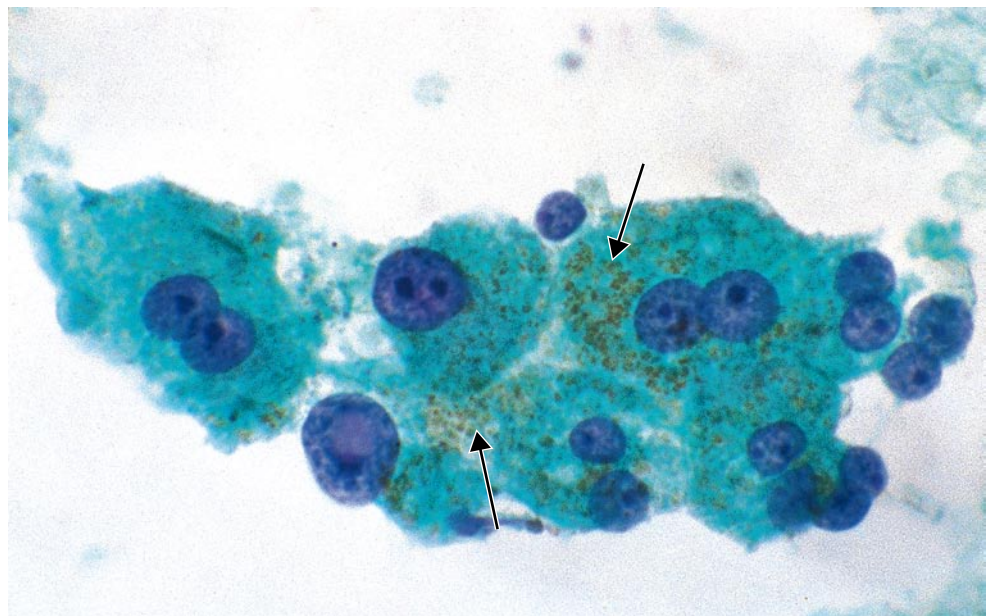


Figure 12.2 Bile pigment. With the Diff-Quik stain, bile pigment is prominent as dense, dark greenish-black pigment in the cytoplasm of hepatocytes (arrow). This case is from a patient with cirrhosis. Bile pigment is usually not seen in normal hepatocytes (Diff-Quik stain).

The first four conditions listed in the differential diagnosis have characteristic clinical and radiographic findings and should be considered when an FNA of the liver is composed entirely of normal liver cells (predominantly hepatocytes) and the patient has a focal lesion. The possibility of sampling error must also be

Figure 12.1 Normal hepatocytes. Hepatocytes have abundant granular cytoplasm, a round and regular nucleus (or two), and a prominent nucleolus. Here they are arranged as a ribbon of two cells' width. Lipofuscin, the normal “wear and tear” pigment, is present (arrow; Papanicolaou stain).



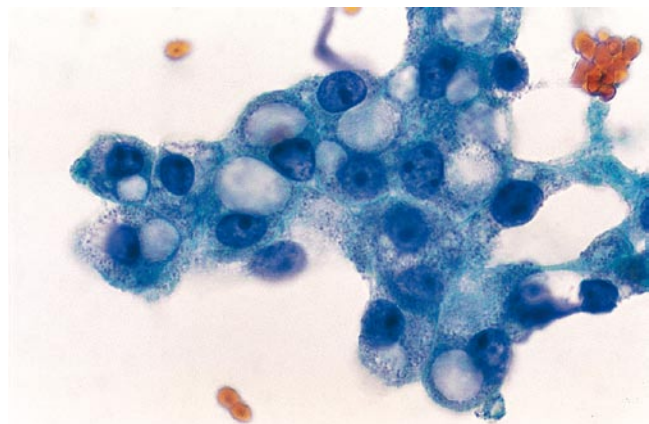


Figure 12.3 Steatosis. This patient had a longstanding history of alcohol abuse, and diffuse nodularity was noted on computed tomography (CT). Fine-needle aspiration (FNA) demonstrated abundant fatty changes (Papanicolaou stain).

considered. In steatosis (fatty metamorphosis), many hepatocytes have large cytoplasmic vacuoles filled with lipid (Fig. 12.3). This alteration of the liver is seen with toxic-metabolic injuries such as those caused by alcohol consumption, diabetes, obesity, drugs (e.g., methotrexate), total parenteral nutrition, postjejunoileal bypass surgery, and hepatitis C. Although usually a diffuse liver abnormality, some cases of steatosis show areas of low attenuation on CT, which suggest an infiltrative tumor.

INFECTIONS

Hepatic Abscess

Hepatic abscesses can be bacterial, fungal, or amebic (principally as a result of *Entamoeba histolytica*). Bacterial abscesses result from ascending cholangitis and sepsis. The most common organisms are streptococci, staphylococci, and enteric bacteria. Fungal abscesses are most common in patients who are immunocompromised; *Candida* species are the most common pathogens.

CYTOMORPHOLOGY OF BACTERIAL AND FUNGAL ABSCESES:

- abundant polymorphonuclear leukocytes and necrotic debris
- may see bacteria and fungi in routine stains, but special stains (i.e., silver or gram) and culture are helpful

Amebic abscesses are rare in the United States but common in other countries, particularly developing nations. They are caused by the protozoan *E. histolytica* and are usually a sequela of colonic infection.

CYTOMORPHOLOGY OF AMEBIC ABSCESS:

- “anchovy paste” (necrotic debris)
- little if any acute inflammation
- amebic trophozoites resemble histiocytes:
 - round nucleus
 - peripheral chromatin margination
 - abundant ovoid cytoplasm containing ingested red blood cells

A careful search for malignant cells is essential whenever an FNA shows abundant necrotic debris, acute inflammation, or both. Microbiologic culture helps to confirm a presumptive diagnosis of infection. For this reason, an on-site evaluation is crucial to ensure that aspirated material is sent for culture.

Echinococcal Cyst (Hydatid Cyst)

The larval form of *Echinococcus granulosus*, a dog tapeworm, causes infection in a variety of organs in humans, principally the liver. In one series of hepatic cysts greater than or equal to 4 cm in diameter, 10% were echinococcal cysts.⁴³ The disease is endemic in the countries bordering the Mediterranean and Baltic seas, in South America, and in Australia and New Zealand. It is also seen in North America. Infection may be asymptomatic. Imaging studies reveal a solitary cyst, often with a fluid level. An outer, acellular, laminated membrane lines the cyst. The internal, germinal layer gives rise to daughter cysts, each of which contains scolices with numerous hooklets. Hooklets resist degeneration but scolices can be lost in longstanding cysts with degeneration.

CYTOMORPHOLOGY OF ECHINOCOCCAL CYST:

- fragments of the laminated membrane (Fig. 12.4)
- scolices
- hooklets (see Fig. 12.4 inset)

Although anaphylactic shock has been reported as an occasional complication of FNA,³⁹ its incidence has not been established, and successful aspiration without serious complications has been noted in other studies.^{29,44,45} Other parasitic diseases that present as a liver mass include schistosomiasis and clonorchiasis.

Other Infections

Infection by the hepatotropic viruses (hepatitis A, B, and C) is generally not evaluated by FNA because the diagnostic changes of acute, subacute, and chronic hepatitis caused by these viruses are primarily architectural and not cytologic. Patients who are immunocompromised

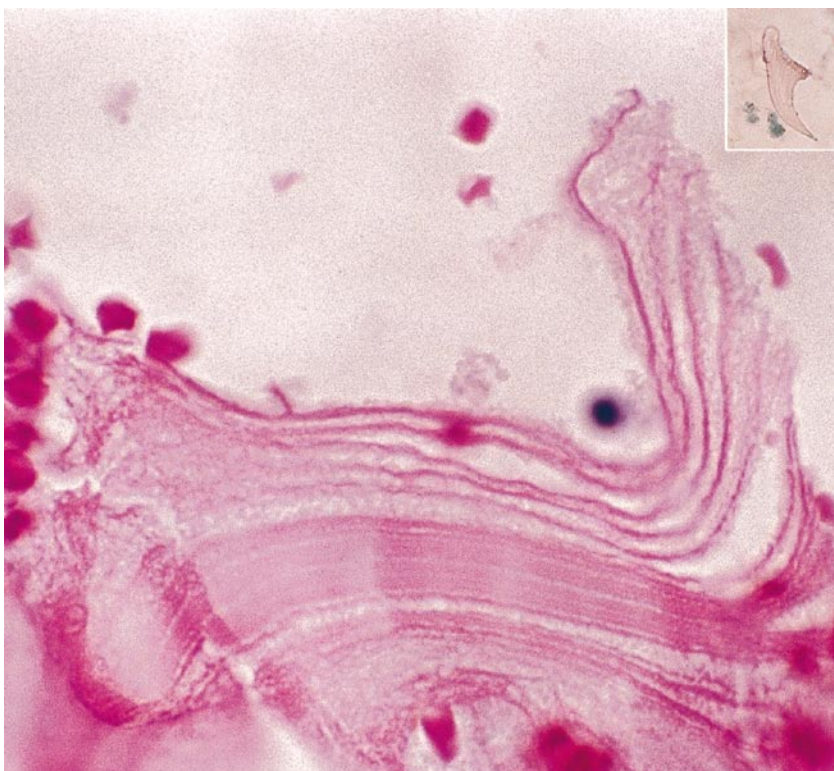


Figure 12.4 Echinococcal cyst. Fragments of the laminated membrane of this organism appear as parallel, acellular striations and are diagnostic (hematoxylin-eosin [H & E]-stained cell block). *Inset:* Some cysts contain mostly acellular debris without scolices. A diligent search uncovers the pale dagger-like hooklets, which do not stain with the Papanicolaou stain (Papanicolaou stain).

may develop hepatitis caused by cytomegalovirus and herpes viruses, but these also result in diffuse liver disease and are rarely diagnosed by FNA.

Granulomas are seen in miliary tuberculosis, sarcoidosis, primary biliary cirrhosis, Hodgkin lymphoma, and drug reactions. Cytologic features are described elsewhere (see Figs. 2.15 and 11.5). The differential diagnosis of granulomatous inflammation includes a hepatic angiomatoma (AML) because the myoid cells of an AML have a syncytium-like appearance similar to that of epithelioid histiocytes. The presence of adipocytes and extramedullary hematopoiesis are a clue to the diagnosis of AML.

BENIGN LESIONS

Solitary Cysts

Solitary, nonparasitic cysts of the liver include the simple unilocular cyst, which is lined by cuboidal or columnar epithelium that resembles biliary epithelium. The aspirated fluid is typically hypocellular and non-diagnostic.

The ciliated foregut cyst is also solitary and unilocular and is lined by respiratory-type epithelium.

CYTOMORPHOLOGY OF CILIATED FOREGUT CYST:

- ciliated columnar cells
- mucus cells

DIFFERENTIAL DIAGNOSIS OF CILIATED FOREGUT CYST:

- bile duct cystadenoma
- bile duct cystadenocarcinoma

Bile duct cystadenomas and cystadenocarcinomas are multilocular tumors lined by mucinous epithelium and filled with watery or viscous fluid. The cystadenoma is lined by a single layer of benign mucin-producing cells, whereas the cystadenocarcinoma shows nuclear pleomorphism and stromal infiltration; the latter cannot be evaluated on cytologic preparations.

Cirrhosis

Cirrhosis, whether caused by alcoholic hepatitis, viral hepatitis, or other diseases, results in a disruption of normal liver architecture, with bands of fibrosis separating nodules of regenerating hepatocytes. Some regenerative nodules are larger than others, and on imaging studies, raise the specter of malignancy, primarily that of HCC. This is compounded by the fact that patients with cirrhosis are at increased risk for developing HCC. Patients with focal lesions in the setting of cirrhosis are often biopsied by FNA, although accuracy may be higher with needle biopsy or a combination of the two.²

CYTOMORPHOLOGY OF CIRRHOSIS:

- normal-appearing hepatocytes, sometimes with steatosis (see Fig. 12.3)
- focal atypia in some cases
 - marked variation in nuclear size
 - prominent nucleolus
 - binucleation

DIFFERENTIAL DIAGNOSIS OF CIRRHOSIS:

- HCC
- hepatic adenoma
- FNH
- normal liver
- nodular regenerative hyperplasia

When hepatocyte atypia is the result of cirrhosis, smears show a wide morphologic spectrum ranging from normal hepatocytes to markedly atypical ones, whereas smears from an HCC are generally monomorphic. Other features common in HCC and rare in cirrhosis and other benign diseases are an increased nuclear-to-cytoplasmic ratio, a trabecular arrangement of hepatocytes surrounded by endothelial cells, and atypical naked nuclei. Using a combination of features permits the diagnosis of most cases of HCC,^{22,46} and dysplastic small cells may be more useful in diagnosing HCC than larger cells.⁴⁷ Cirrhosis can be distinguished from HCC by FNA, with larger tissue fragments in cirrhosis and microtrabeculae of irregular thickness in HCC.⁴⁸ The differential diagnosis of HCC is covered in depth later in this chapter.

Specimens composed of hepatocytes without atypia are cytologically indistinguishable from the remainder of the conditions listed in the differential diagnosis; the diagnosis requires clinical-pathologic correlation. Nodular regenerative hyperplasia (nodular transformation of the liver) is a poorly understood condition in which small nodules of regenerating liver are scattered diffusely throughout the liver. Unlike cirrhosis, these nodules are not separated by bands of fibrosis, but patients often have portal hypertension and ascites.

Focal Nodular Hyperplasia

FNH is a benign liver lesion that possibly results from focal vasculopathy. It usually presents as a solitary mass or, less commonly, several masses. Most patients are women in their third or fourth decade. The nodules usually contain a central scar, which can be appreciated on imaging studies. Histologic examination reveals nodules of hepatocytes that are separated by radiating fibrous septae that contain bile ductules.

CYTOMORPHOLOGY OF FOCAL NODULAR HYPERPLASIA:

- hepatocytes without significant atypia (Fig. 12.5)
- bile ductular cells

DIFFERENTIAL DIAGNOSIS OF FOCAL NODULAR HYPERPLASIA:

- hepatic adenoma
- regenerating nodules in cirrhosis
- normal liver

These lesions are cytologically indistinguishable, and radiologic and clinical correlation is necessary.

Liver Cell Adenoma

Liver cell adenomas are uncommon benign neoplasms usually encountered in women under the age of 30, especially those who have a history of long-term use of oral contraceptives. Patients often present with abdominal pain.⁴⁹ These lesions may rupture through the liver capsule, resulting in intraperitoneal hemorrhage. Histologically, they lack portal triads and are composed exclusively of hepatocytes.

CYTOMORPHOLOGY OF LIVER CELL ADENOMA:

- predominance of hepatocytes
- mild nuclear atypia (Fig. 12.6)
- abundant cytoplasm
- normal nuclear-to-cytoplasmic ratio

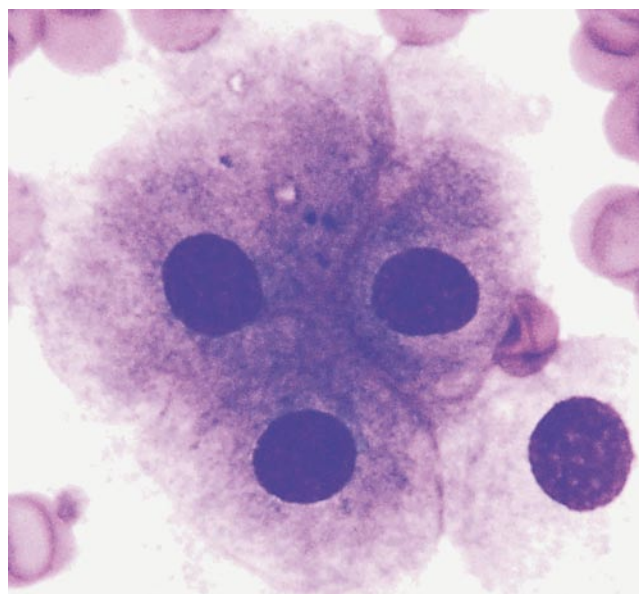


Figure 12.5 Focal nodular hyperplasia (FNH). These cells are indistinguishable from normal hepatocytes. A nonspecific negative diagnosis was made (Diff-Quik stain).

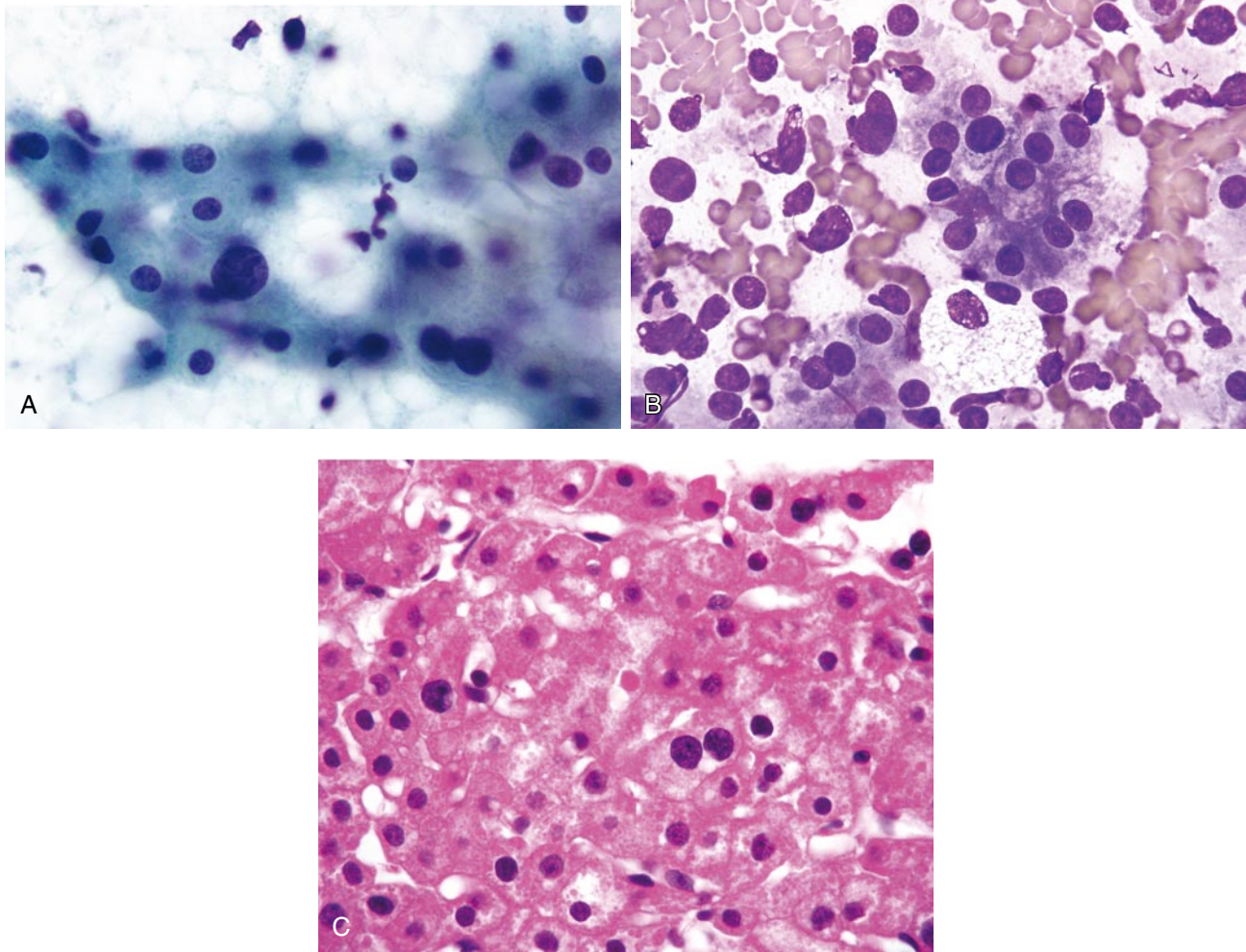


Figure 12.6 Liver cell adenoma. On high-power view, there is only mild nuclear atypia, with slight variation in cell size and some binucleated cells. In contrast, the cells of hepatocellular carcinoma (HCC; see Fig. 12.10B) demonstrate greater cellular atypia. A, Papanicolaou stain, B, Diff-Quik stain, C, cell block, hematoxylin-eosin (H & E) stain.

DIFFERENTIAL DIAGNOSIS OF LIVER CELL ADENOMA:

- normal liver
- FNH
- regenerating nodules in cirrhosis
- HCC

Although it has been suggested that the absence of bile duct epithelium supports a diagnosis of hepatic adenoma and excludes FNH,⁵⁰ for all practical purposes cytomorphology alone cannot distinguish between an adenoma, FNH, and cirrhosis. In practice, clinical and radiologic correlation is necessary. The cells of HCC have a higher nuclear-to-cytoplasmic ratio and more nuclear atypia than seen in adenomas.

Bile Duct Hamartoma and Adenoma

The bile duct hamartoma (von Meyenburg complex) is characterized by multiple small nodules dispersed throughout the liver and composed of haphazardly arranged bile ductules and fibrous stroma. The bile duct adenoma is usually a solitary subcapsular nodule less than 1 cm in diameter.

CYTOMORPHOLOGY OF BILE DUCT HAMARTOMA AND ADENOMA:

- hypocellular specimens
- benign ductal cells in tubules and cohesive sheets
- benign hepatocytes

Hemangioma

The hemangioma is the most common benign tumor of the liver. Histologically, dilated vascular spaces are lined by benign endothelial cells. Most hemangiomas are recognized radiologically with sufficient accuracy and an FNA is not necessary. Occasional hemangiomas have unusual imaging findings, however, and an FNA is performed to exclude a more significant lesion. If well sampled, cytologic findings are characteristic. Some cases, however, show only blood or benign hepatocytes.⁵¹ If a spindle cell lesion of the liver is encountered, it is most likely a hemangioma. The next most likely lesions are metastatic leiomyosarcoma and granulomatous hepatitis.⁵²

CYTOMORPHOLOGY OF HEMANGIOMA:

- blood only (some cases)
- hepatocytes only (some cases)
- three-dimensional arcades of bland elongated spindle cells
- compact, dense coils of spindle cells (Fig. 12.7A)
- rare isolated spindle-shaped cells
- vascular channels lined by endothelial cells (cell block sections; Fig. 12.7B).

Angiomyolipoma

AML is the most common benign tumor of the kidney. It is seen less often in other locations, but it does occur in the liver, mediastinum, heart, spermatic cord, vaginal wall, fallopian tube, oral cavity, pharynx, nasal cavity, skin, and parotid gland. Of the extrarenal sites, the liver is the most common; to date, over 100 hepatic AMLs have been reported worldwide.⁵³

Hepatic AML shares many clinical features with its renal counterpart. The average age at diagnosis is about

50 years. Although 60% of patients are symptomatic, many tumors, particularly small ones, are diagnosed incidentally by imaging studies. Hepatic AMLs can rupture spontaneously, just like their renal counterparts, but this is uncommon. Some patients with a hepatic AML have tuberous sclerosis as do some patients with a renal AML.

AMLs of the liver range in size from 0.3 to 36 cm in diameter (mean 9 cm) and are well-circumscribed. Hemorrhage, necrosis, and calcification are rare. As with AMLs of the kidney, many are not biopsied because their high fat content permits an accurate diagnosis by CT or MRI studies. Only the radiologically atypical AMLs (usually the result of low fat content) undergo biopsy. One study of 49 sporadic hepatic AMLs found no loss of heterozygosity or microsatellite instability in any of the cases.⁵⁴

The proportions of the components vary considerably from patient to patient. Some tumors are composed predominantly of adipose tissue, whereas others have virtually no fat and are almost exclusively smooth muscle. The myoid cells may cause diagnostic difficulty because they can be epithelioid, may be markedly pleomorphic with bizarre giant cells, and may show mitotic activity. In contrast to renal AMLs, extramedullary hematopoiesis is a frequent feature.

CYTOMORPHOLOGY OF HEPATIC ANGIOMYOLIPOMA:

- clusters of epithelioid or spindle cells (myoid cells; Fig. 12.8)
- fat cells
- blood vessels
- extramedullary hematopoiesis (40%) (see Fig. 12.8)

Hepatic AMLs can be diagnosed accurately by FNA.⁵⁵ The correct diagnosis rests on identifying the triad of fat, vessels, and smooth muscle, but the myoid component is

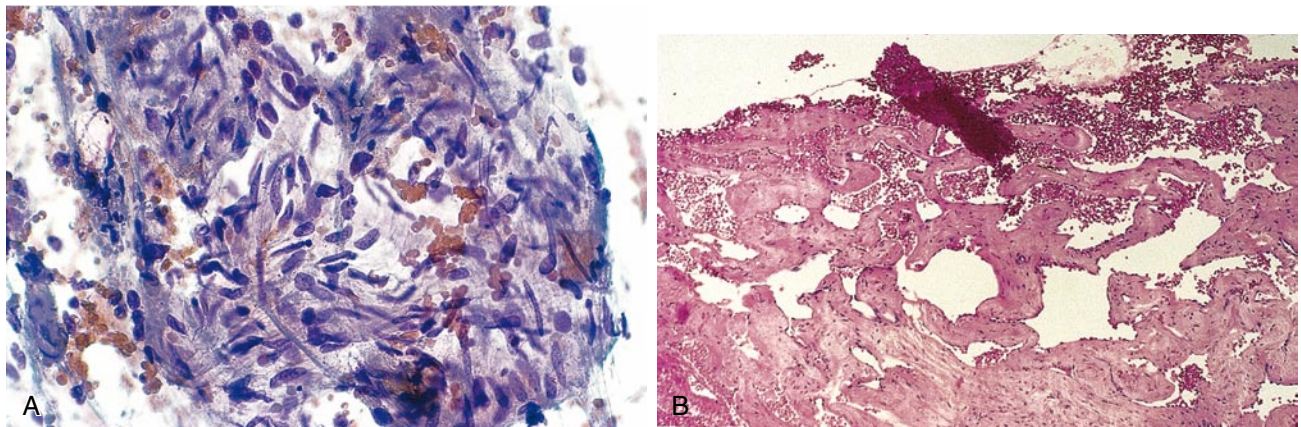


Figure 12.7 Hemangioma. A, Smears show occasional tangles of spindle-shaped cells that are difficult to classify (Papanicolaou stain). B, The diagnosis is easier with cell block sections, in which the vascular architecture is more readily apparent (hematoxylin-eosin [H & E] stain).

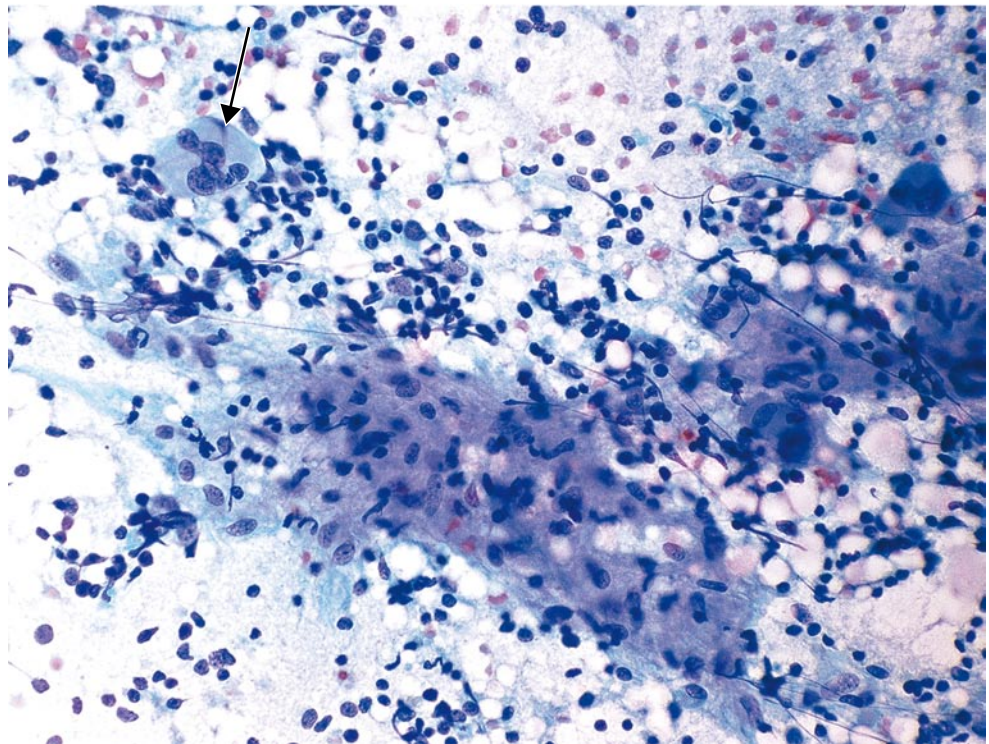


Figure 12.8 Angiomyolipoma (AML) of the liver. The myoid cells are sometimes epithelioid, as in this case, and have indistinct cell borders. Note the prominent component of extramedullary hematopoiesis, including megakaryocytes (arrow; Papanicolaou stain).

the only specific and diagnostic component of AML. The myoid cells have indistinct cell membranes and abundant granular cytoplasm. Sheets of myoid cells, therefore, have a syncytium-like appearance that resembles that of a granuloma. Adipose tissue is not consistently present.⁵⁶ The consistent positivity of the myoid cells for HMB-45 is a helpful diagnostic feature (see Fig. 14.4D). Almost one half of AMLs of the liver have extramedullary hematopoiesis, a striking finding that should make one consider the diagnosis of an AML.

DIFFERENTIAL DIAGNOSIS OF HEPATIC ANGIOMYOLIPOMA:

- HCC
- myelolipoma of the liver
- nodular hematopoiesis
- sarcomas
- other carcinomas
- granulomatous inflammation

The differential diagnosis includes HCC. The cells of HCC have distinct cytoplasmic borders, unlike the myoid cells of AML, which have a fibrillar cytoplasm with indistinct cell membranes. Because hematopoietic elements are seen in many hepatic AMLs, the differential diagnosis also includes myelolipoma and nodular hematopoiesis of the liver, but cytologic samples from these lesions lack the myoid cells of AML. Because the myoid cells vary widely in their appearance, they can mimic a variety of spindle cell and epithelioid cell

neoplasms of the liver. Their indistinct cell membranes and arrangement in nodular aggregates may cause them to be mistaken for the epithelioid histiocytes of granulomas, especially when hematopoietic elements are interspersed. Ultimately, the diagnosis rests on showing that the large neoplastic cells are *positive* for HMB-45.

Because a small percentage of hepatic AMLs rupture, a patient who is symptomatic or has a large tumor or does not have a definitive diagnosis may be considered for resection. Nevertheless, the majority of patients are spared surgical resection, and their tumors are monitored by periodic imaging studies.

MALIGNANT TUMORS

Hepatocellular Carcinoma

HCC accounts for 90% of all primary cancers of the liver. It is less common in the United States and Western Europe than in Africa and Asia. In the United States, the majority of cases are seen in the setting of cirrhosis. Most patients are over the age of 50, but children and younger adults can be affected. The tumor can present as a solitary nodule, as multiple nodules, or as diffuse liver enlargement. Histologically, HCCs show trabecular, tubular, and solid growth patterns. There is a wide range of differentiation, from well-differentiated tumors that resemble normal liver, to poorly differentiated ones with marked nuclear pleomorphism and tumor giant cells. Serum

levels greater than 4000 ng/mL of alpha-fetoprotein (AFP) are highly suggestive of HCC, but elevated titers are not present in all cases. Because the differential diagnosis at either end of the spectrum (i.e., well or poorly differentiated HCC) is challenging, these will be presented separately.

CYTOMORPHOLOGY OF WELL-DIFFERENTIATED HEPATOCELLULAR CARCINOMA:

- highly cellular smears with isolated cells or cells in cords, nests, tubules, or sheets (Fig. 12.9)
- spindle-shaped endothelial cells surround thickened cords of neoplastic hepatocytes (Figs. 12.10, 12.11)
- increased nuclear-to-cytoplasmic ratio (Fig. 12.12)
- granular cytoplasm with bile or hyaline globules (red with Papanicolaou and blue with Romanowsky stains; Figs. 12.13, 12.14)
- large, round nucleus with prominent nucleolus
- intranuclear pseudoinclusions (Fig. 12.15)
- large naked nuclei (Fig. 12.16)

DIFFERENTIAL DIAGNOSIS OF WELL-DIFFERENTIATED HEPATOCELLULAR CARCINOMA:

- regenerating nodules of cirrhosis
- hepatic adenoma
- FNL

Thickened trabeculae of hepatocytes surrounded by endothelial cells, isolated and atypical naked nuclei, and

an increased nuclear-to-cytoplasmic ratio are characteristic of HCC.^{46,57-62} Cellular monomorphism, nuclear crowding, macronucleoli, multinucleated cells, mitoses, and loss of bile duct cells are also typical of HCC, as are capillaries traversing tissue fragments; some features are better seen on cell block sections than on smears.^{5,61,63} Endothelium, either wrapped around thickened trabeculae or transgressing sheets of cells, is particularly useful.^{64,65} The context (thickened trabeculae) and abundance of the wrapping endothelium are important, inasmuch as over one half of benign aspirates contain tissue fragments with focal but identifiable endothelial cells.⁶⁶ The presence of endothelial cells around cell fragments should alert one to the possibility of HCC, but it should not be used in isolation. Other criteria such as nuclear atypia and size of the fragments should also be evaluated. Irregularly arranged, overlapping cells in sheets are also typical of HCC, in contrast with the evenly spaced arrangement of benign lesions.⁶⁷

CD34 reactivity may be useful in diagnosing HCC as compared to cirrhosis; however, significant reactivity with FNH and hepatic adenoma limits its use.⁶⁸⁻⁷⁰ AFP and polyclonal carcinoembryonic antigen (CEA) are also useful in this setting.⁷⁰ Decreased or absent reticulin, with expanded trabeculae and islands or sheets, is seen in HCC, whereas benign lesions have a normal trabecular reticulin pattern.^{61,69,71,72} Other markers for separating benign and malignant liver cells include CK18, glypican 3 (GPC3),⁷³⁻⁷⁷ and alpha-methylacyl-CoA racemase AMACR (P504S).^{78,79}

Other approaches to separate HCC from regenerative or benign conditions include the use of proliferation markers such as proliferating cell nuclear antigen (PCNA), nucleolar organizer regions, and DNA content.⁸⁰⁻⁸⁵ Immunoreactivity for mutant p53 expression increases with increasing grades of HCC.⁸⁵⁻⁸⁷

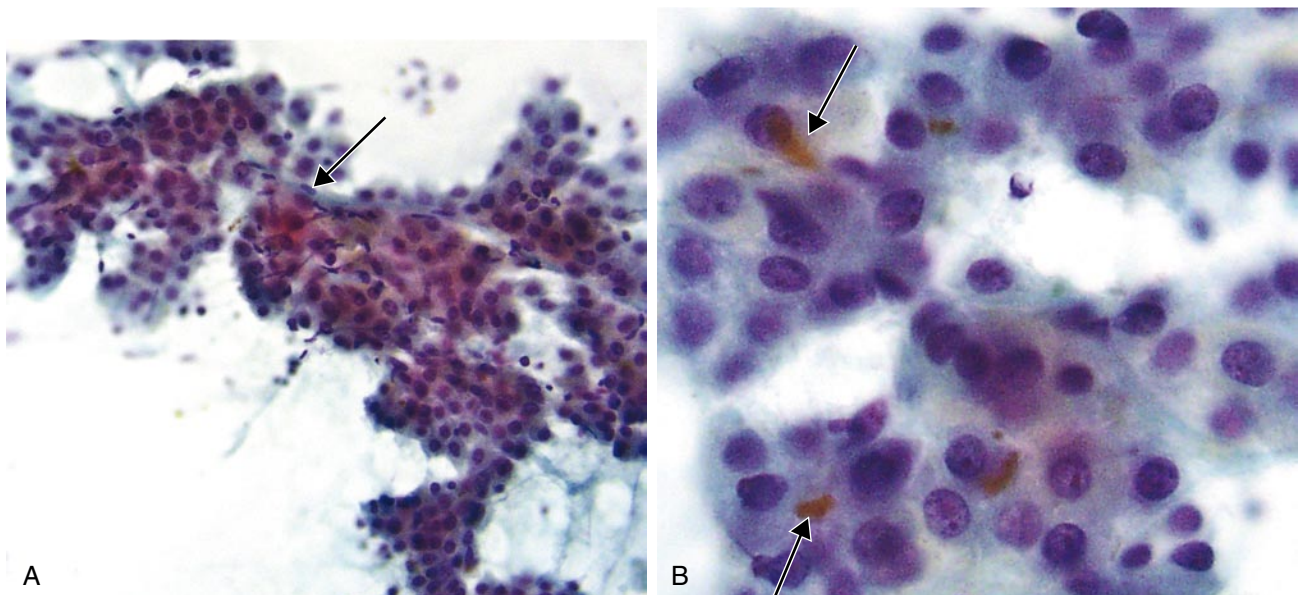


Figure 12.9 Hepatocellular carcinoma (HCC). A, On low power, the aspirate is hypercellular and endothelial cells can be appreciated coursing through the aggregates of hepatocytes. B, Bile is prominent even on low-power views (Papanicolaou stain).

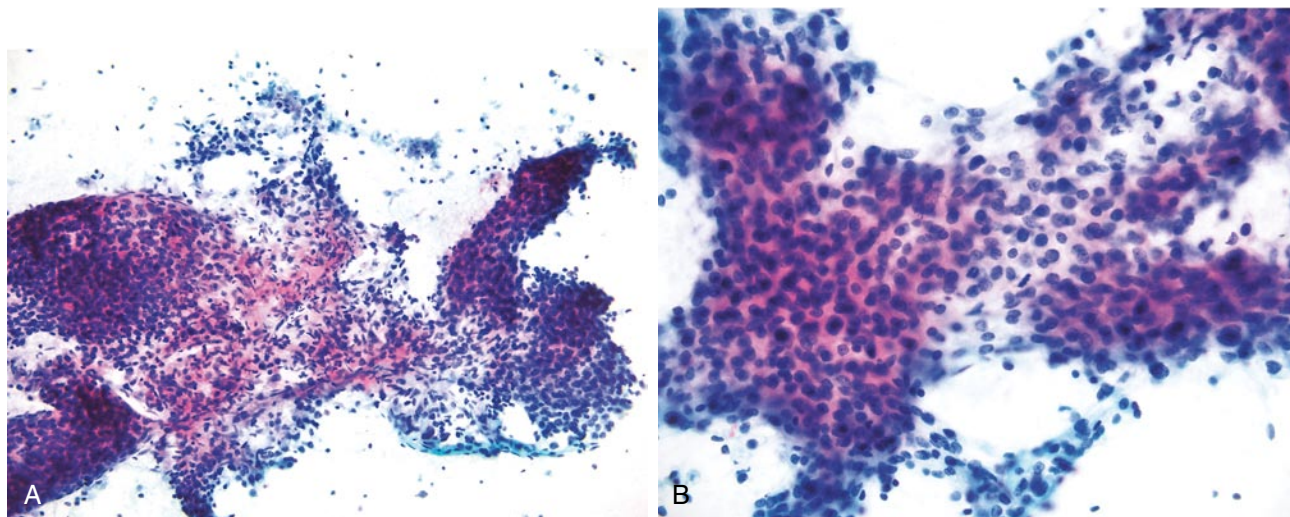


Figure 12.10 Hepatocellular carcinoma (HCC). A, Markedly thickened trabeculae of neoplastic cells are surrounded by and intertwined with endothelial cells (Papanicolaou stain). B, At higher power, the increased nuclear to cytoplasmic ratio and mitoses are recognized (Papanicolaou stain).

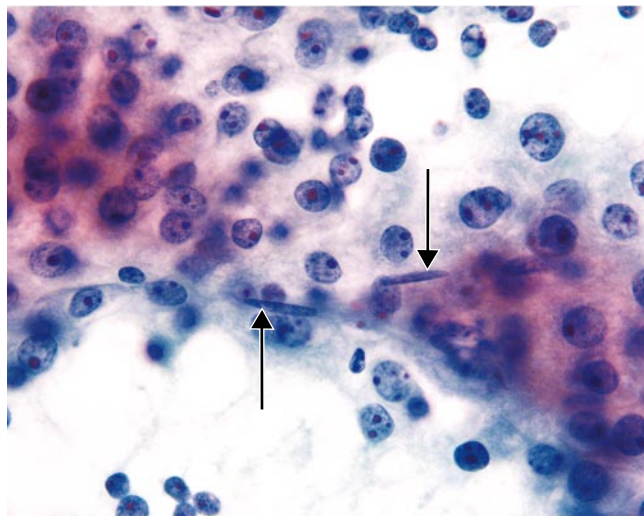


Figure 12.11 Hepatocellular carcinoma (HCC). At high power, spindle-shaped endothelial cells are recognized coursing among malignant hepatocytes (Papanicolaou stain).

CYTOMORPHOLOGY OF MODERATELY AND POORLY DIFFERENTIATED HEPATOCELLULAR CARCINOMA:

- highly cellular smears (see Fig. 12.16)
- single cells or cords, nests, tubules, or sheets
- moderate to marked pleomorphism (see Figs. 12.16, 12.17)
- atypical mitoses
- spindled-shaped cells
- tumor giant cells (Figs. 12.18 and 12.19)
- clear cytoplasm in about 10% of HCC (“clear cell HCC”), resembling renal and adrenocortical carcinoma

DIFFERENTIAL DIAGNOSIS OF MODERATELY AND POORLY-DIFFERENTIATED HEPATOCELLULAR CARCINOMA:

- cholangiocarcinoma
- metastatic carcinoma

Poorly differentiated HCC is difficult to distinguish from cholangiocarcinoma and metastatic carcinomas by cytologic features alone, especially because some HCCs have a tubular pattern. In addition, there are rare cases of combined HCC and cholangiocarcinoma that present a special diagnostic challenge.⁸⁸⁻⁹⁰ Rare HCCs have abundant finely vacuolated to clear cytoplasm mimicking renal, adrenal, or ovarian origin; immunohistochemical studies are necessary.^{91,92} Hyaline globules are seen in HCC, but they should also raise the suspicion of renal cell carcinoma.⁹³

The most notable features of HCC are polygonal cell shape with a centrally placed nucleus, cytoplasmic bile, and sinusoidal capillaries separating neoplastic cells, all of which are common in HCC and rare in other carcinomas.⁵⁸ Architectural features in cell block material are useful as well, particularly the characteristic sinusoidal spaces and trabecular arrangement of HCC.⁹⁴

Special stains are helpful in the distinction between primary and metastatic liver tumors (see Table 12.1). Intracytoplasmic mucin is extremely uncommon in HCC but present in most adenocarcinomas, including cholangiocarcinoma. Polyclonal CEA is also useful. With this antibody, many cases of HCC show a distinctive linear staining pattern outlining bile canaliculi (Fig. 12.20A); this is not seen with adenocarcinomas, which show diffuse cytoplasmic staining.⁹⁵⁻¹⁰¹ Other useful antibodies for metastatic carcinoma include Cu-18 (BCA-225),

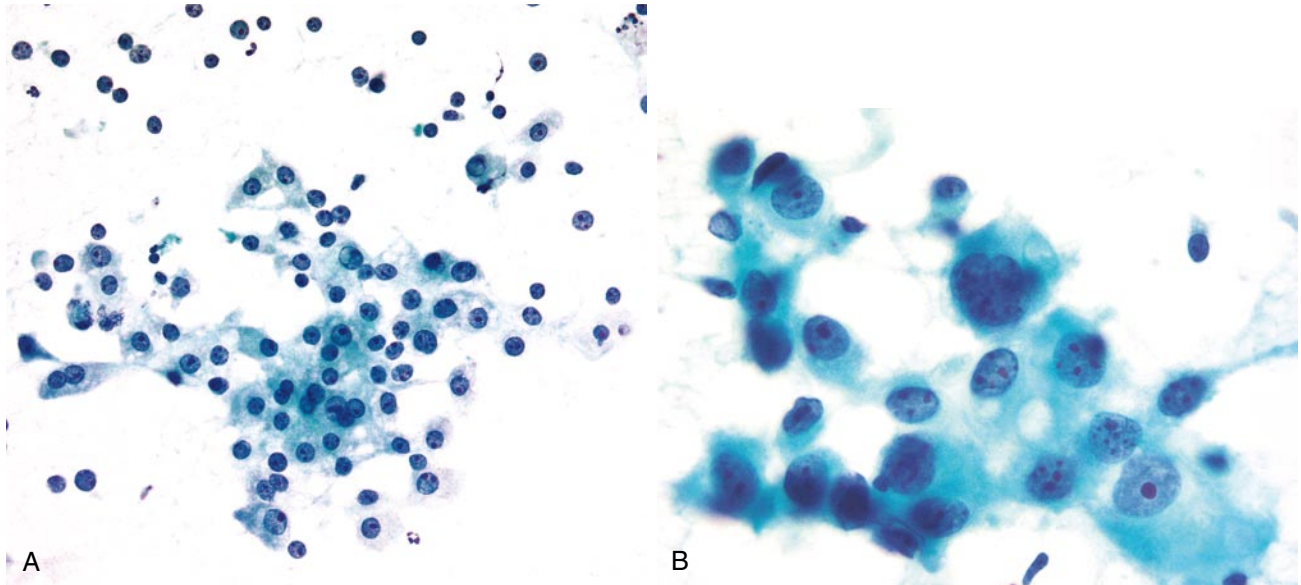


Figure 12.12 Hepatocellular carcinoma (HCC). A, B, The aspirate is markedly hypercellular. There is a pleomorphic population of cells. The cells resemble normal hepatocytes, but the nuclear-to-cytoplasmic ratio is increased and there are numerous naked nuclei (Papanicolaou stain).

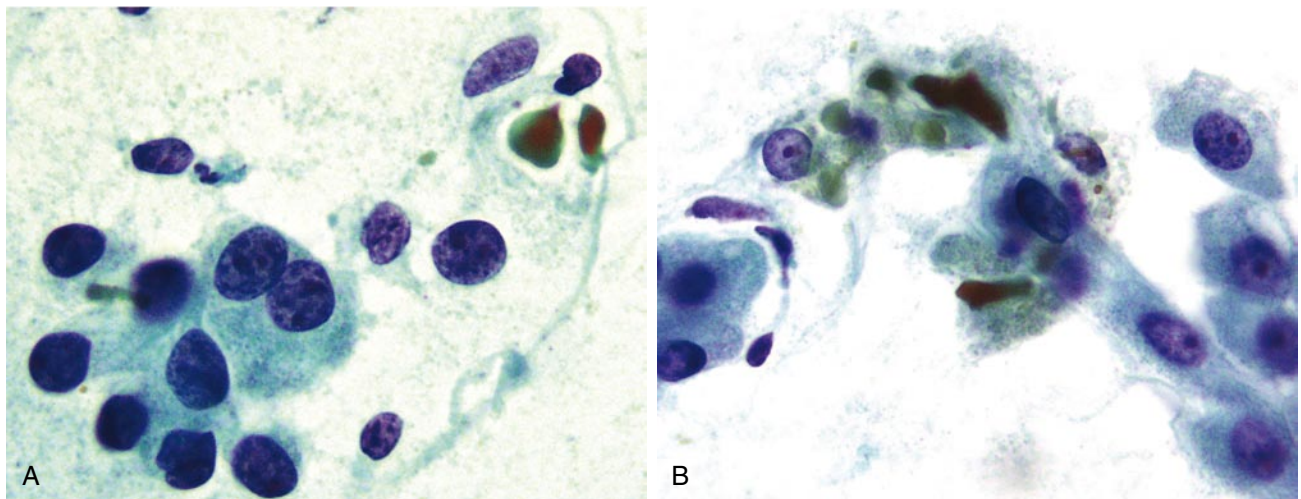


Figure 12.13 Hepatocellular carcinoma (HCC). A, Bile pigment is sometimes prominent, as in this case. B, Both bile and endothelial cells are noted (Papanicolaou stain).

monoclonal antibody discriminating between mesothelial and epithelial cells (MOC-31), and anion exchanger 1 and 3 (AE1/AE3).^{95,97,101,102} About one half of HCC cases are positive for hepatitis B viral antigens; this finding is rare in cholangiocarcinoma and absent in metastatic carcinomas.¹⁰³ Hepatocyte Paraffin 1 (HepPar1) antibody has high sensitivity and specificity for HCC (Fig. 12.20B), but there may not be uniform staining.^{70,96,104,105} HepPar1 staining may also be seen in tumors other than HCC.^{70,105,106} The use of this antibody with MOC-31, polyclonal and monoclonal CEA, and CD10 has been suggested as a useful panel to separate HCC and metastatic carcinoma in cytology specimens.^{107,108} Other markers for hepatic origin include cytokeratin CAM 5.2,

CK8, and CK18 and stains that help to exclude hepatic origin, including CK7, CK19, CK20, or AE1/AE3. CK8 or CK18 usually stains in HCC, but not in cholangiocarcinoma, which is more likely to have CK7, CK17, and CK19 staining,¹⁰⁹ although these can occasionally be seen in some HCCs.¹¹⁰

Thyroid transcription factor-1 (TTF-1) stains normal hepatocytes and HCCs in a granular cytoplasmic (rather than nuclear) staining pattern and is a useful marker of hepatocystic differentiation (Fig. 12.20C).¹¹¹⁻¹¹³ The cytoplasmic staining of TTF-1 appears to be mitochondrial, similar to that of HepPar1.¹¹⁴ Immunostains for AFP or alpha-1-antitrypsin are not sufficiently sensitive or specific for the diagnosis of HCC.⁹⁵

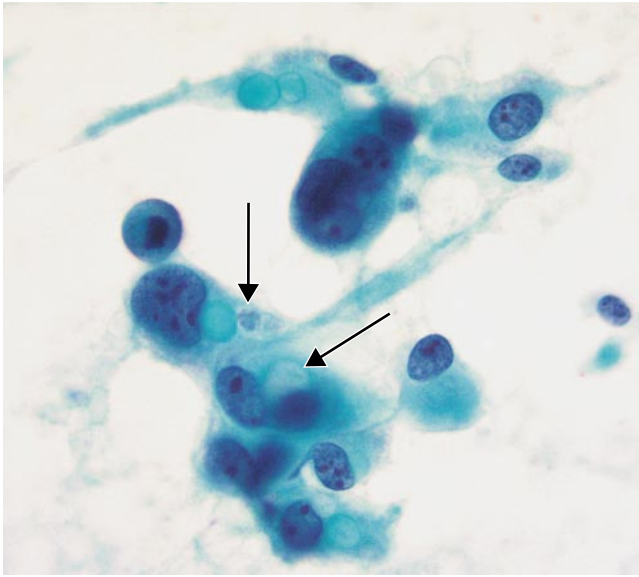


Figure 12.14 Hepatocellular carcinoma (HCC). There are pleomorphic isolated cells and a tumor giant cell. The cytoplasm is granular, with prominent intracytoplasmic vacuoles containing hyaline globules (arrow; Papanicolaou stain).

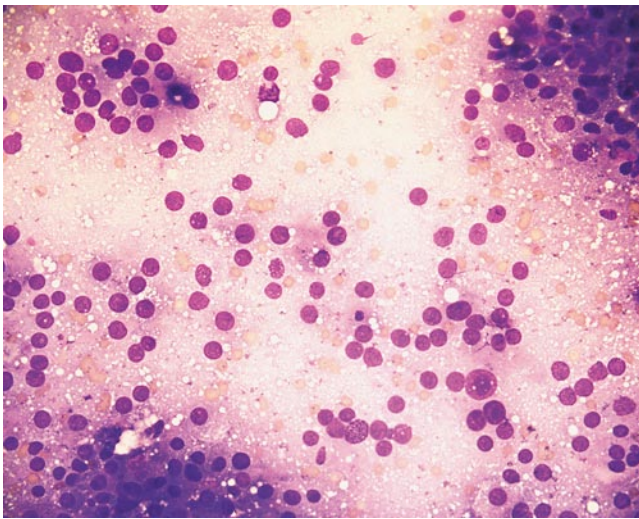


Figure 12.16 Hepatocellular carcinoma (HCC). This low-power photograph demonstrates marked hypercellularity. Nuclear pleomorphism can be demonstrated even at this power. Bare, pleomorphic nuclei are numerous. This picture should stimulate review of high power features to confirm malignancy (Diff-Quik stain).

CD34 and factor VIII antibody are useful for highlighting the wrapping endothelial cells of HCC.^{59,115} Numerous spider-shaped vimentin-positive Kupffer cells are noted in HCCs and not in metastatic carcinomas.¹¹⁶

The combination of HepPar1 and a monoclonal antibody of epithelial antigen (BerEP4) has been suggested as the first step in an algorithm to separate HCC from regenerative nodules and metastatic carcinoma.¹¹⁷ If HepPar1 is strongly positive (>20% of cells) and BerEP4 is negative (<20% of cells), then AFP, nuclear antigen Ki-67, CD31,

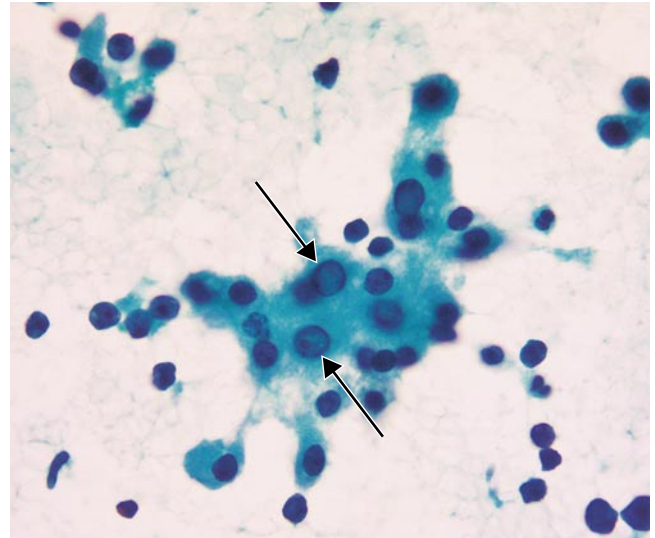


Figure 12.15 Hepatocellular carcinoma (HCC). This loosely cohesive cluster of cells shows many cells with prominent intranuclear vacuoles (arrow; Papanicolaou stain).

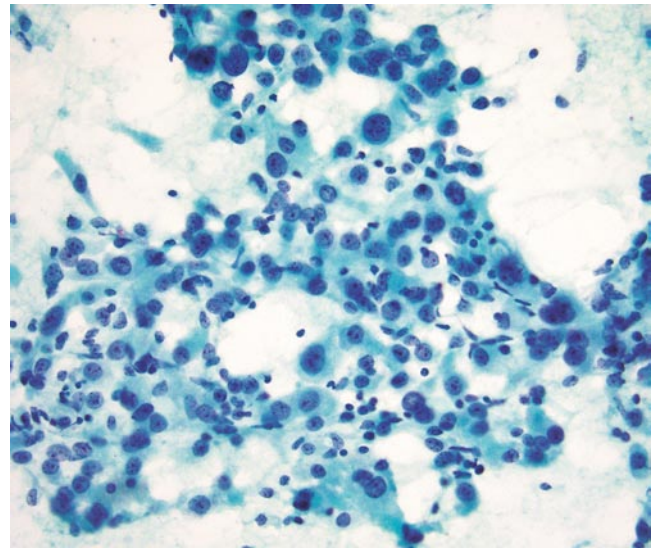


Figure 12.17 Hepatocellular carcinoma (HCC). There is a loosely cohesive cluster of atypical hepatocytes with intermingled endothelial cells. Nuclear pleomorphism is noted. Binucleated and multinucleated cells are seen. Nucleoli are multiple and often irregular (Papanicolaou stain).

and CD68 are used to separate HCC from regenerative nodules.¹¹⁷ If, however, HepPar1 is negative (no staining) and BerEP4 is positive (>20% of cells), then CK7 and CK20 are the next step in determining the site of origin: CK 7 > CK20 staining in lung and ovaries; CK7 = CK20 in stomach and pancreas; and CK7 < CK20 in colon. Finally, one or more the following antibodies is applied: TTF-1 (lung) caudal-related homeobox 2 ([CDX-2] stomach, pancreas, ovaries, colon), cancer antigen 125 ([CA125] ovaries), and CK5 and CK6 (stomach/pancreas).¹¹⁷

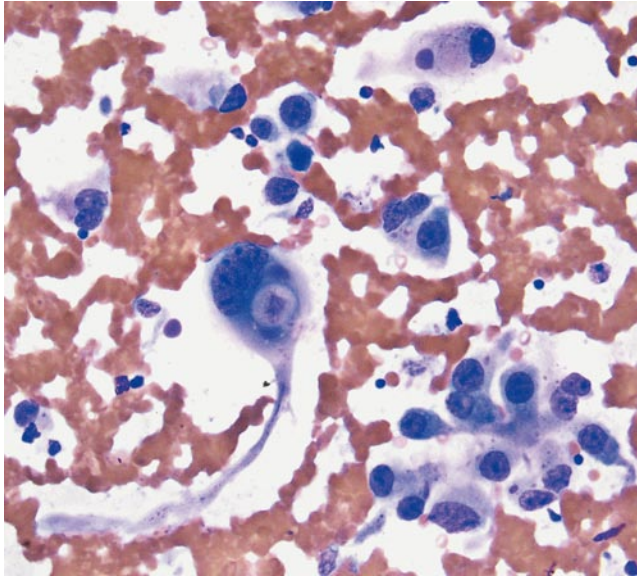


Figure 12.18 Hepatocellular carcinoma (HCC). Bizarre atypical forms including spindle cells with intracytoplasmic globules are noted (Diff-Quik stain).

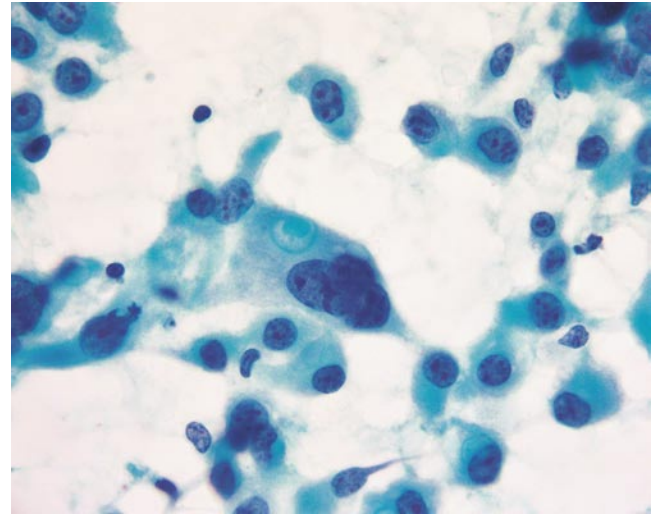


Figure 12.19 Hepatocellular carcinoma (HCC). Multinucleated tumor cells are common. Such cells have three or more nuclei in contrast to benign hepatocytes that are commonly binucleated (Papanicolaou stain).

TABLE 12.1 SPECIAL STAINS IN THE DIFFERENTIAL DIAGNOSIS OF LIVER TUMORS

	Hepatocellular Carcinoma	Cholangiocarcinoma	Metastatic Adenocarcinoma
Bile	+/-	-	-
Mucin	-	75%–100%	50%–75%
AE1 keratin	rare	+	+
Polyclonal CEA	canalicular	diffuse cytoplasmic	diffuse cytoplasmic
HBV	50%	rare	-
HepPar1	80%	50%	rare
CD31	+ (endothelial cells)	-	-
TTF-1	granular, cytoplasmic	-	nuclear (lung)
Ber-EP4	-	+	+
MOC-31	-	+	+
Combination			
CD10	canalicular	—	-
CD34	sinusoidal	—	+/-
monoclonal CEA	-	—	+
CK7	-	+	variable (+) lung, ovaries, stomach, pancreas; (-) in colon
CK20	-	+/- (=40%–50%)	variable (+) in stomach, pancreas and colon; (-) in lung and ovaries
CDX-2	-	rare (20%)	+ stomach, pancreas, and colon
CK8/18	+	-	—
CK 5/6	-	rare (20%)	+ in stomach and pancreas
CK19	rare	+	—
CK17	-	+	—

AE1, low-molecular-weight keratin anion exchanger 1; Ber-EP4, monoclonal antibody of epithelial antigen; CEA, carcinoembryonic antigen; CK, cytokeratin; HBV, hepatitis B viral antigen; HepPar1, Hepatocyte Paraffin 1; MOC-31, monoclonal antibody discriminating between mesothelial and epithelial cells; TTF-1, thyroid transcription factor 1.

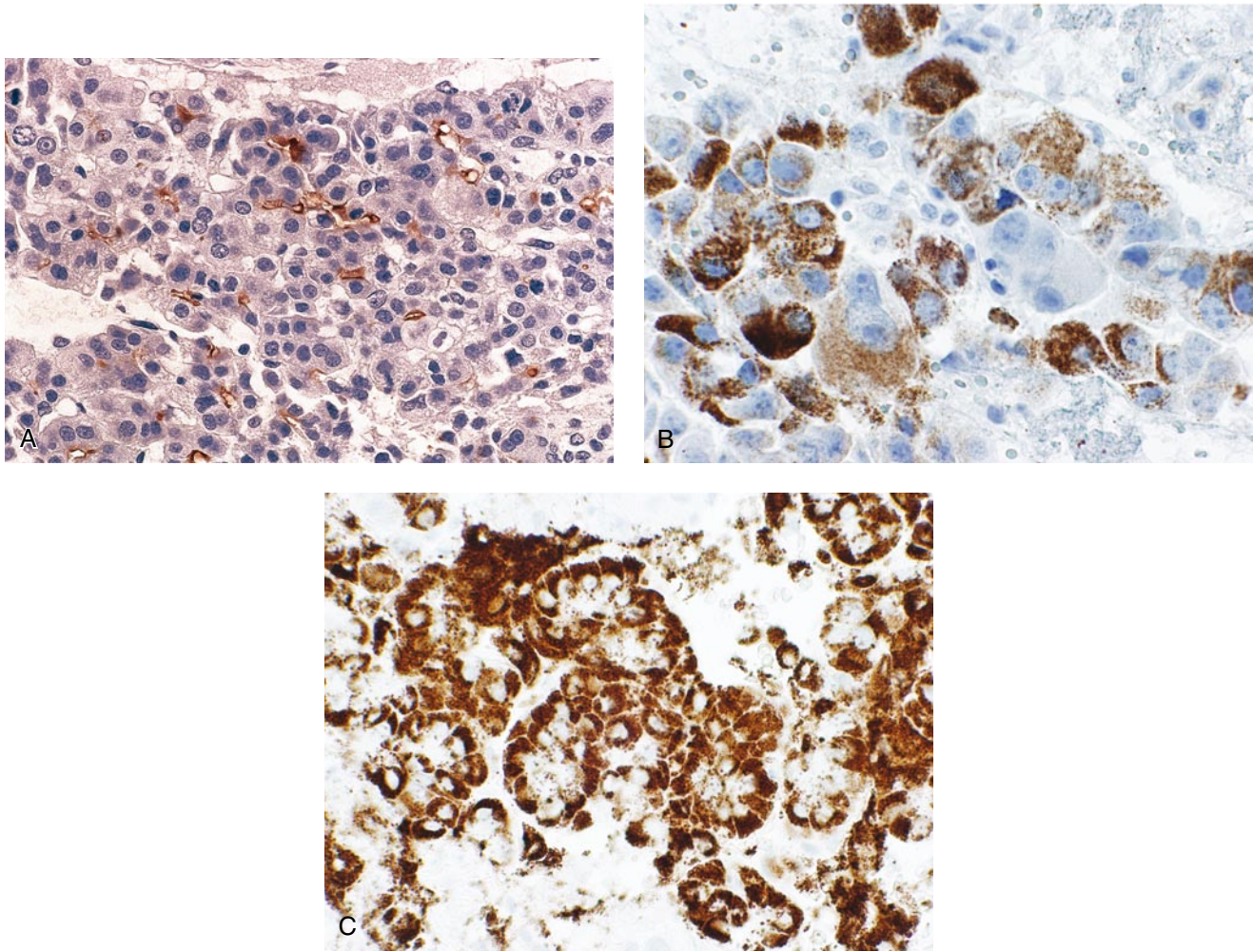


Figure 12.20 Immunoprofile of hepatocellular carcinoma (HCC). A, Immunoreactivity with polyclonal antibodies to carcinoembryonic antigen (CEA) leaves the malignant cells unstained but outlines the bile canaliculi as short, discontinuous linear threads and small lumina. B, Most HCCs are immunoreactive for Hepatocyte Paraffin 1 (HepPar1). C, Most HCCs show cytoplasmic immunoreactivity for thyroid transcription factor-1 (TTF-1).

The fibrolamellar variant of HCC is seen predominantly in young patients (average age, mid-20s) who do not have cirrhosis. Their prognosis is favorable. Histologically, the malignant cells are large and polygonal and contain abundant eosinophilic cytoplasm. Dense bands of fibrosis surround tumor cells.

CYTOMORPHOLOGY OF FIBROLAMELLAR HEPATOCELLULAR CARCINOMA:

- large cells with abundant cytoplasm
- large nucleus
- prominent nucleolus
- mostly isolated cells or loose clusters
- hyaline intracytoplasmic globules (some cells)
- bands of fibrosis separating neoplastic cells (Fig. 12.21)

The cells of fibrolamellar HCC are much larger than normal hepatocytes and larger than the cells of well-

differentiated HCC (see Fig. 12.21).¹¹⁸ Notably, the nuclear-to-cytoplasmic ratio is lower in fibrolamellar HCC than in ordinary HCC.¹¹⁸ In contrast to typical HCC, fibrolamellar HCC and pediatric HCCs are more likely to stain positively with CK7 than adult HCCs.¹¹⁹⁻¹²¹

Cholangiocarcinoma (Bile Duct Carcinoma)

Cholangiocarcinoma is much less common than HCC and is not associated with cirrhosis. Tumors arise from intrahepatic and extrahepatic bile ducts. Obstructive jaundice is the most common presentation in patients with extrahepatic tumors. Significant elevation of carbohydrate antigen 19-9 (Ca19-9) or CEA can be seen. The diagnosis can be made in 60% to 70% of patients using bile cytology, brush cytology, or percutaneous FNA.^{122,123} Increasingly, endoscopic ultrasound-guided FNA (EUS-FNA) is used to diagnose this tumor.¹²⁴⁻¹²⁸

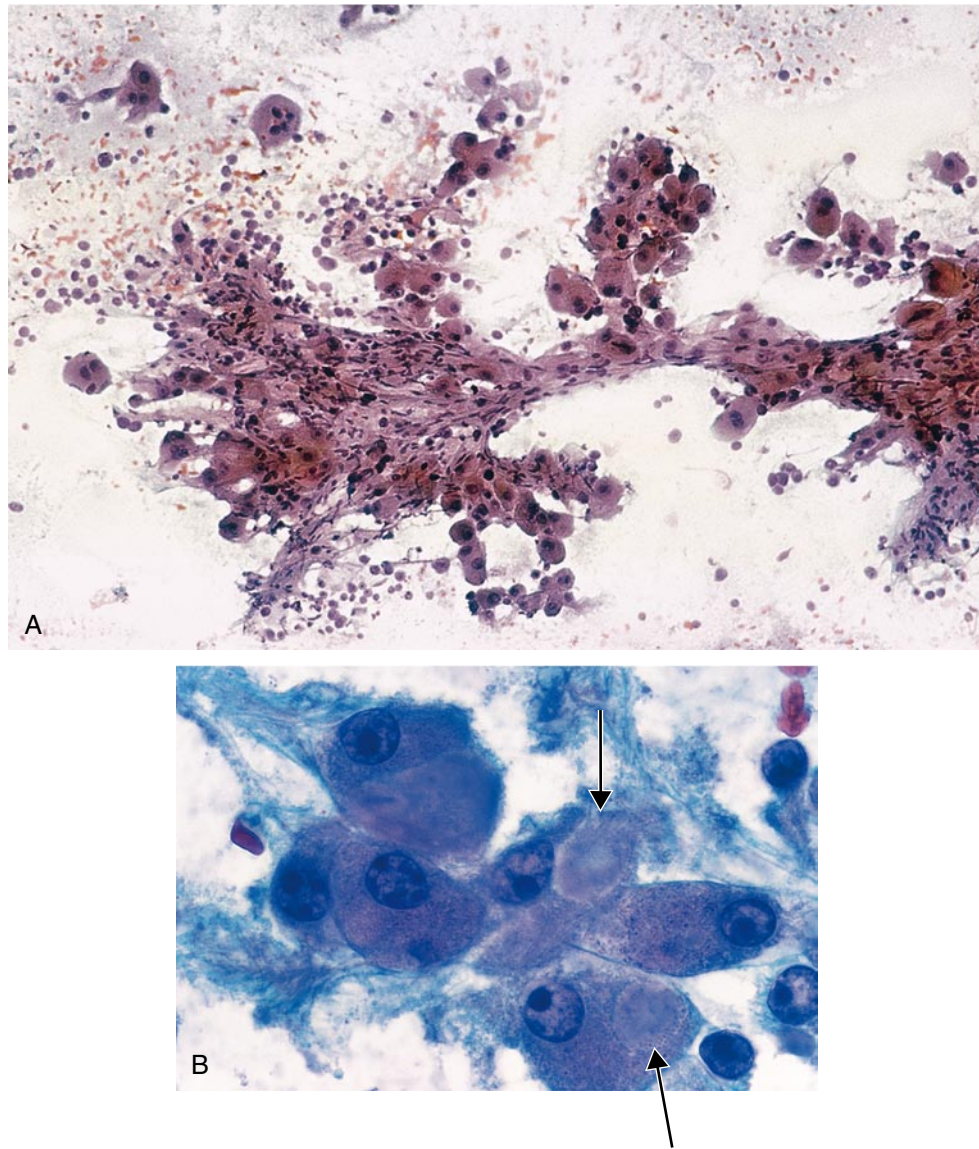


Figure 12.21 Fibrolamellar hepatocellular carcinoma (HCC). A, Large, atypical hepatocytes are accompanied by fibrous tissue. B, The malignant cells have abundant granular cytoplasm and intracytoplasmic hyaline globules (arrows; Papanicolaou stain).

CYTOMORPHOLOGY OF CHOLANGIOCARCINOMA:

- isolated cells, crowded sheets, and cell clusters, some in acinar arrangements (Fig. 12.22A)
- nuclear enlargement with variation in size and shape (Fig. 12.22B)
- scant cytoplasm
- benign hepatocytes

DIFFERENTIAL DIAGNOSIS OF CHOLANGIOCARCINOMA:

- HCC
- metastatic adenocarcinoma

Neither bile nor sinusoidal capillaries separating groups of neoplastic cells, typical of HCC, are seen with cholangiocarcinoma. The cells of most cholangiocarcinomas are cuboidal or columnar rather than polygonal as in HCC. Special stains are quite useful. Cholangiocarcinoma is a gland-forming tumor; mucin production may be abundant.¹²⁹ Positive staining for mucicarmine, AE1 keratin, and diffuse cytoplasmic staining with polyclonal CEA are characteristic of cholangiocarcinoma and are uncommon in HCC. Other immunostains typically staining in cholangiocarcinoma but less commonly in HCC include CK7, CK17, and CK19, whereas HCC (not cholangiocarcinoma) typically stains with CK8 or CK18.¹⁰⁹ The bile canicular staining pattern with polyclonal CEA is seen in some HCCs and

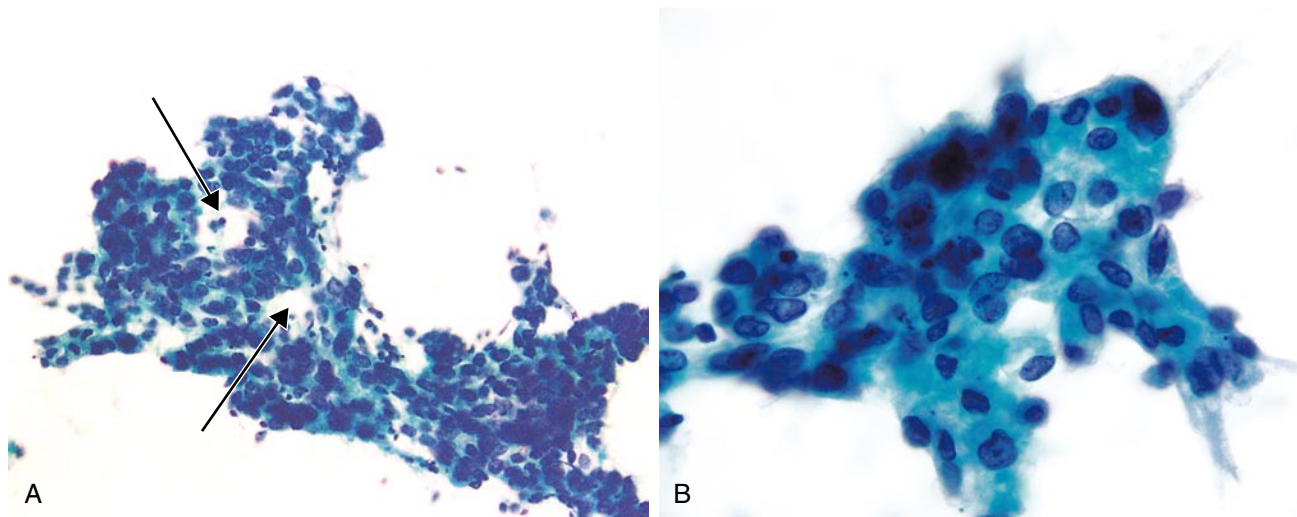


Figure 12.22 Cholangiocarcinoma. A, The tumor cells are present in crowded sheets and clusters. The irregular cellular arrangement and acinar structures are apparent (arrows; Papanicolaou stain). B, The cells are haphazardly arranged in clusters with marked crowding, hyperchromasia, increased nuclear-to-cytoplasmic ratio, and irregularity of nuclear membranes (Papanicolaou stain).

not in cholangiocarcinoma. Hepatitis B viral antigens are uncommon in cholangiocarcinoma.

The distinction between cholangiocarcinoma and metastatic adenocarcinoma is virtually impossible by cytology and immunohistochemistry. The presence of proliferating ductules, in particular more than 10 ductular clusters, has been suggested as a cytologic feature to distinguish cholangiocarcinoma from metastatic adenocarcinoma.¹³⁰ In general, this distinction is best made on clinical grounds by ruling out other primary sites.

Hepatoblastoma

Hepatoblastoma is a rare tumor of infancy and childhood. Cytologic smears mimic those from HCC, with larger and more anaplastic cells, or those from small, blue, round-cell tumors of childhood.¹³¹⁻¹³³

Angiosarcoma

Angiosarcoma is an uncommon malignant tumor of endothelial cells that represents less than 1% of primary hepatic malignancies. Most cases arise in adults, but the tumor can occur in persons of any age. Like HCC, angiosarcoma is associated with cirrhosis; about one third of adult cases arise in this setting. It is seen with increased frequency in workers who are exposed to polyvinyl chloride and in patients who received thorium dioxide (Thorotrast) as a radiographic contrast agent. Histologically, these are spindle cell neoplasms that range from well-differentiated tumors with well-formed vascular channels to poorly differentiated, solid tumors. Massive bleeding is a potential complication of FNA.¹³⁴

CYTOMORPHOLOGY OF ANGIOSARCOMA:

- well-differentiated tumors: elongated cells, isolated in tightly cohesive clusters and syncytia
- poorly differentiated tumors: large, spindle-shaped or epithelioid cells, pleomorphic nuclei, multinucleated giant cells
- “rhabdoid” forms in some epithelioid angiosarcomas¹³⁵
- abundant finely or coarsely vacuolated cytoplasm often with intracytoplasmic lumina¹³⁶
- cell blocks useful in demonstrating the characteristic anastomosing vascular pattern

DIFFERENTIAL DIAGNOSIS OF ANGIOSARCOMA:

- epithelioid hemangioendothelioma
- other malignant neoplasms

Angiosarcoma cells are larger, more atypical and more monomorphous than those of an epithelioid hemangioendothelioma, and are more likely to be cohesive.¹³⁷ They also may have a large nucleolus and intracytoplasmic vacuoles.¹³⁷ Positive immunoreactivity for endothelial markers such as CD34^{136,138-140} or CD31^{136,141} is helpful in confirming the diagnosis and ruling out metastatic sarcomas, most commonly leiomyosarcoma.

Epithelioid Hemangioendothelioma

This uncommon tumor arises in the liver and at other sites. Like angiosarcoma, it is a tumor of endothelial cells,

but its course is less aggressive. The cytologic findings described herein may be suggestive.^{137,142} Immunostains for endothelial markers are usually positive.

CYTOMORPHOLOGY OF EPITHELIOID HEMANGIOENDOTHELIOMA:

- hypocellular (some cases)
- isolated, large polymorphous cells with a folded nuclear outline
- occasional intranuclear cytoplasmic inclusions
- frequent binucleated or multinucleated giant cells
- round or irregular nucleoli and prominent nucleoli
- abundant lacy and hematoxyphilic cytoplasm sometimes with intracytoplasmic lumina¹⁴³
- see Figure 4.15

Metastatic Tumors

The liver is a common site for metastasis, and the majority of FNAs of liver masses prove to be metastatic malignancies.¹⁴⁴ The cytologic picture varies depending on the original tumor. Any lesion discovered on FNA that is suspected of being metastatic should be compared to any available histologic or cytologic slides of the original tumor. When there is no known primary, some cytologic features may suggest a specific primary site. Most metastases are carcinomas. Common sites of origin include breast, colon, lung, pancreas, and stomach.

CYTOMORPHOLOGY OF COMMON METASTATIC MALIGNANCIES TO THE LIVER:

- colorectal cancers:
 - “tall (columnar), dark (hyperchromatic), and necrotic” (Fig. 12.23)
- breast cancer and gastric cancer:
 - signet-ring cells
- prostate (Fig. 12.24):
 - microacini
 - prominent nucleoli
 - immunoreactive for prostatic acid phosphatase and prostate-specific antigen
- well-differentiated neuroendocrine tumors (Fig. 12.25):

- eccentrically placed nucleus
- “salt-and-pepper” chromatin pattern
- abundant granular cytoplasm
- isolated cells, loosely cohesive clusters and rosettes
- immunoreactive for neuroendocrine markers¹⁴⁵
- small cell carcinoma (Fig. 12.26):
 - isolated and loosely cohesive cells
 - nuclear molding
 - hyperchromatic nucleus with finely granular chromatin and small nucleoli
 - round, polygonal or spindled cells
- metastatic squamous cell carcinoma (Fig. 12.27):
 - small, dark nucleus without discernible texture
 - abundant cytoplasm with a hard, glassy appearance (orange with Papanicolaou stain and gray with Romanowsky-type stain)
 - more poorly differentiated, nonkeratinized type hard to distinguish from other poorly differentiated malignancies
- malignant melanoma (Fig. 12.28):
 - abundant cytoplasm, sometimes pigmented
 - intranuclear pseudoinclusions and macronucleolus
 - isolated cell pattern without trabeculae or clusters
 - melanin pigment is finely granular (bile pigment more variable in size)
 - immunoreactive for S-100, HMB-45, and Mart-1
- sarcoma (Figs. 12.29, 12.30):
 - spindle-shaped cells
 - differential diagnosis: metastatic sarcomatoid carcinoma (pancreas, lung, gall bladder, and kidney)

Extramedullary hematopoiesis in the liver, seen in patients with myeloproliferative disorders, can be diagnosed when megakaryocytes, erythroid precursors, and myeloid precursors are identified. Lymphomas occur as metastatic or primary tumors of the liver,¹⁴⁶ and features are described in Chapter 11. Germ cell tumors may metastasize to liver. Most show positive immunoreactivity for AFP; so the use of HepPar1 and other markers such as human chorionic gonadotropin is mandatory.

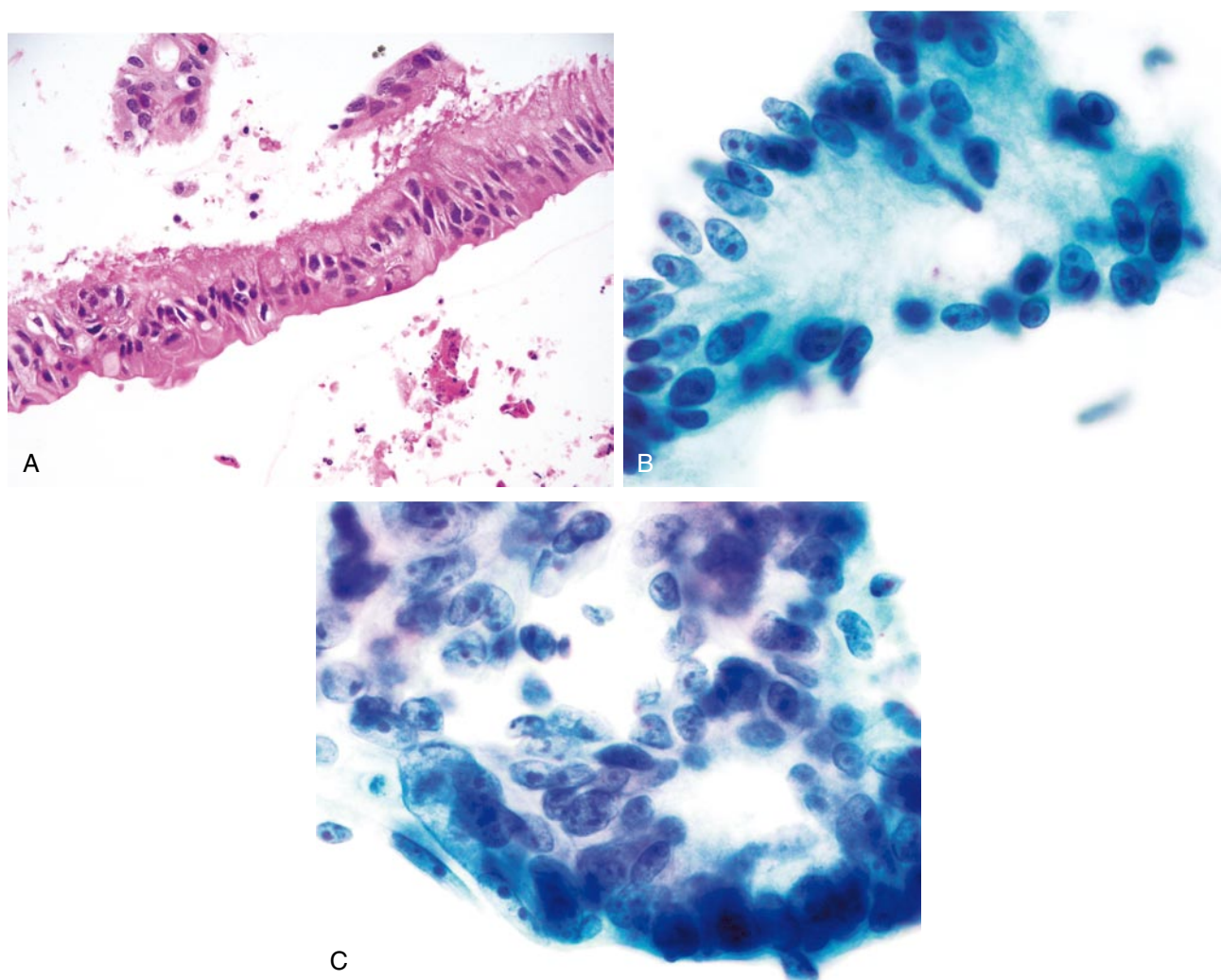


Figure 12.23 Metastatic colon carcinoma. A, Colon cancer can present in a picket fence arrangement with a dirty, necrotic background (cell block, hematoxylin-eosin [H & E] stain). B, At higher power, the picket fence arrangement is noted, and nuclei are enlarged and ovoid (Papanicolaou stain). C, Sheets with acini and pronounced nuclear hyperchromasia are noted. Together these features constitute the classic “tall, dark, and necrotic” picture of this malignancy (Papanicolaou stain).

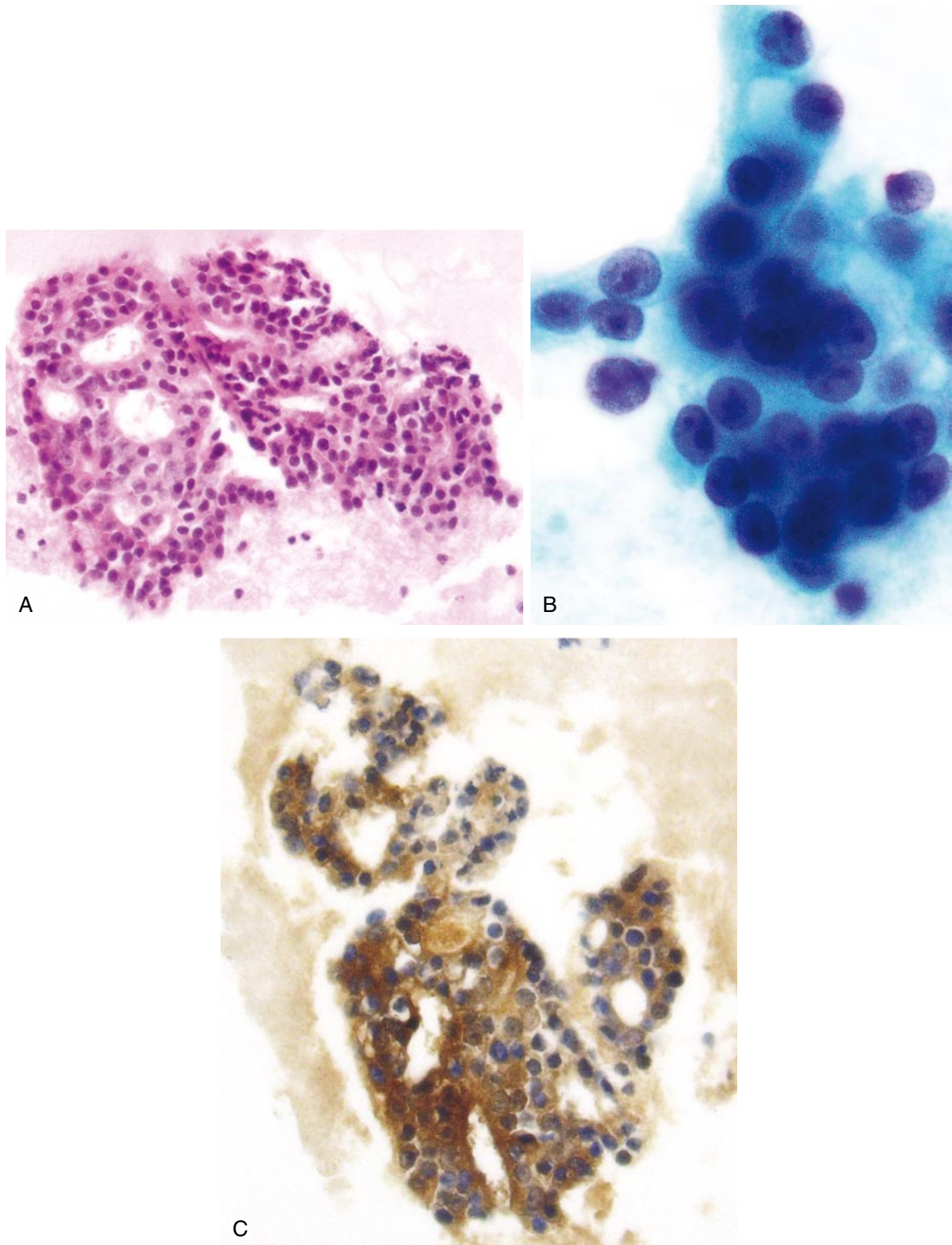


Figure 12.24 Metastatic prostatic carcinoma. A, Acinar structures are common (cell block, hematoxylin-eosin [H & E]). B, Microacinar structures are the most characteristic appearance, and nucleoli can be prominent. (Papanicolaou stain). C, Staining for prostate-specific antigen (PSA) or prostatic acid phosphatase (Papanicolaou not shown) confirms the diagnosis (cell block, immunostain for PSA).

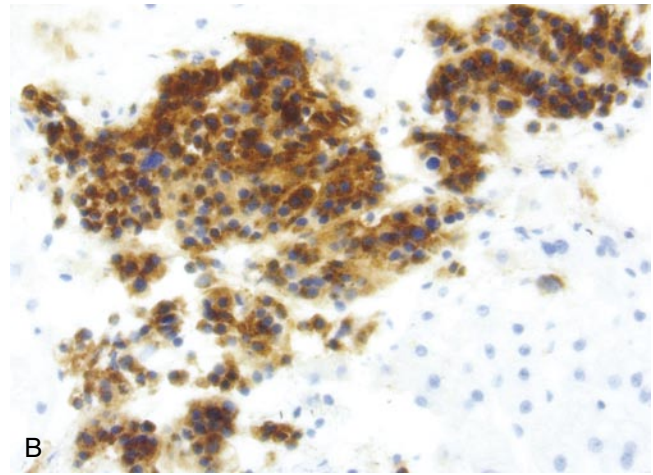
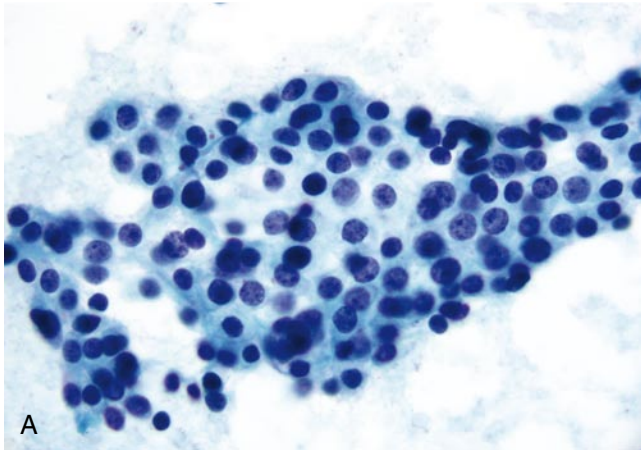


Figure 12.25 Metastatic neuroendocrine tumor (carcinoid). A, The tumor cells are arranged in loose clusters. The nuclei are hyperchromatic with a coarsely granular "salt-and-pepper" pattern (Papanicolaou stain). B, Immunostains for neuroendocrine markers like chromogranin are strongly positive in contrast to surrounding normal hepatocytes (cell block, chromogranin immunostain).

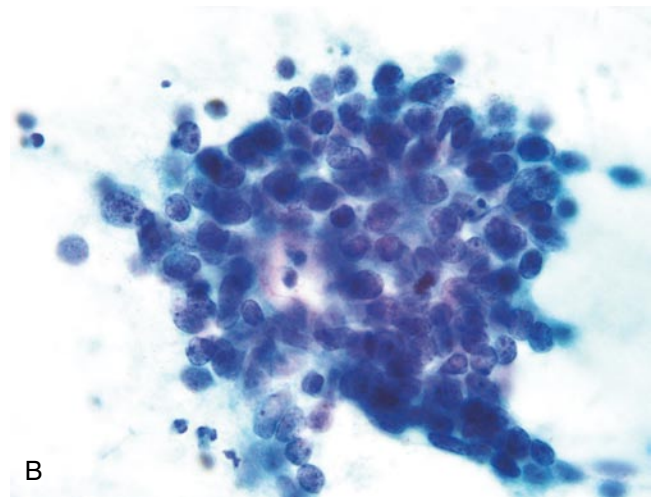
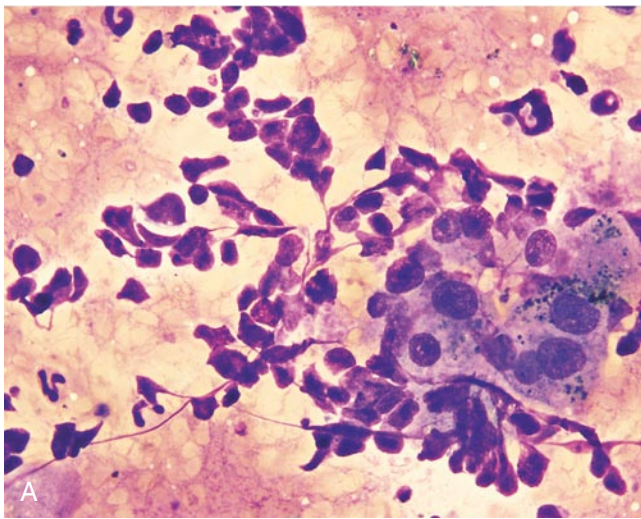


Figure 12.26 Metastatic small cell carcinoma. A, Note the small blue cells with scant cytoplasm and pronounced molding, arrayed around benign hepatocytes. Nuclear crush artifact is prominent (Diff-Quik stain). B, The cells are isolated and arranged in loosely cohesive clusters. The nuclear-to-cytoplasmic ratio is quite high, nuclear molding is seen, and the chromatin is finely granular without nucleoli (Papanicolaou stain).

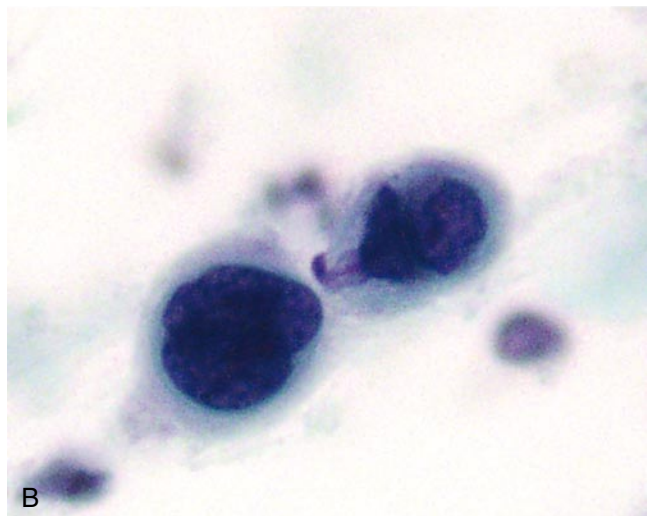
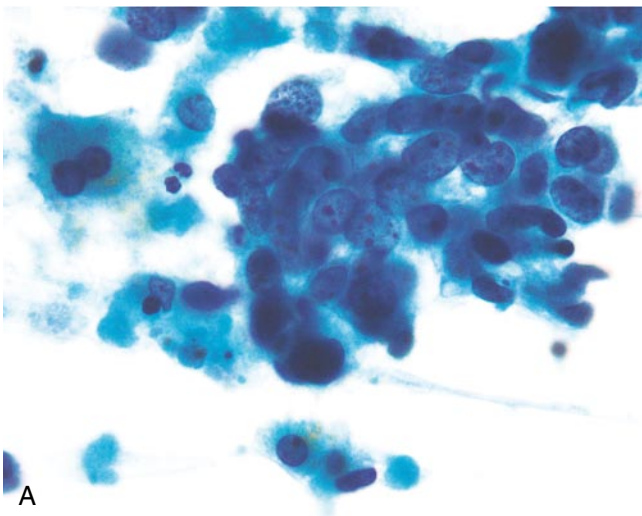


Figure 12.27 Metastatic squamous cell carcinoma of the larynx. A, This tumor is more difficult to distinguish from other primary and metastatic malignancies when keratinization is absent. Finding two distinct populations of cells (i.e., tumor cells and benign hepatocytes) without intermediate forms favors a diagnosis of metastatic carcinoma. B, Isolated cells with hyperchromatic and pyknotic nuclei are present.

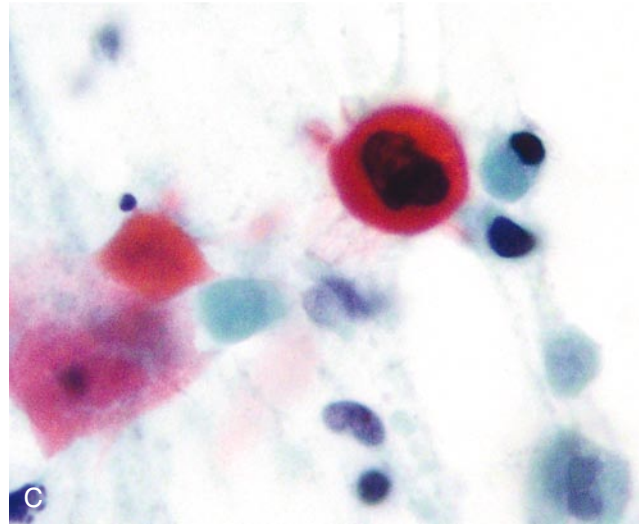


Figure 12.27 (Continued) C, **Dense cytoplasmic eosinophilia or orangeophilia is diagnostic.** To determine the primary site, comparison with slides from the prior malignancy or the application of a panel of immunostains is often necessary (Papanicolaou stain).

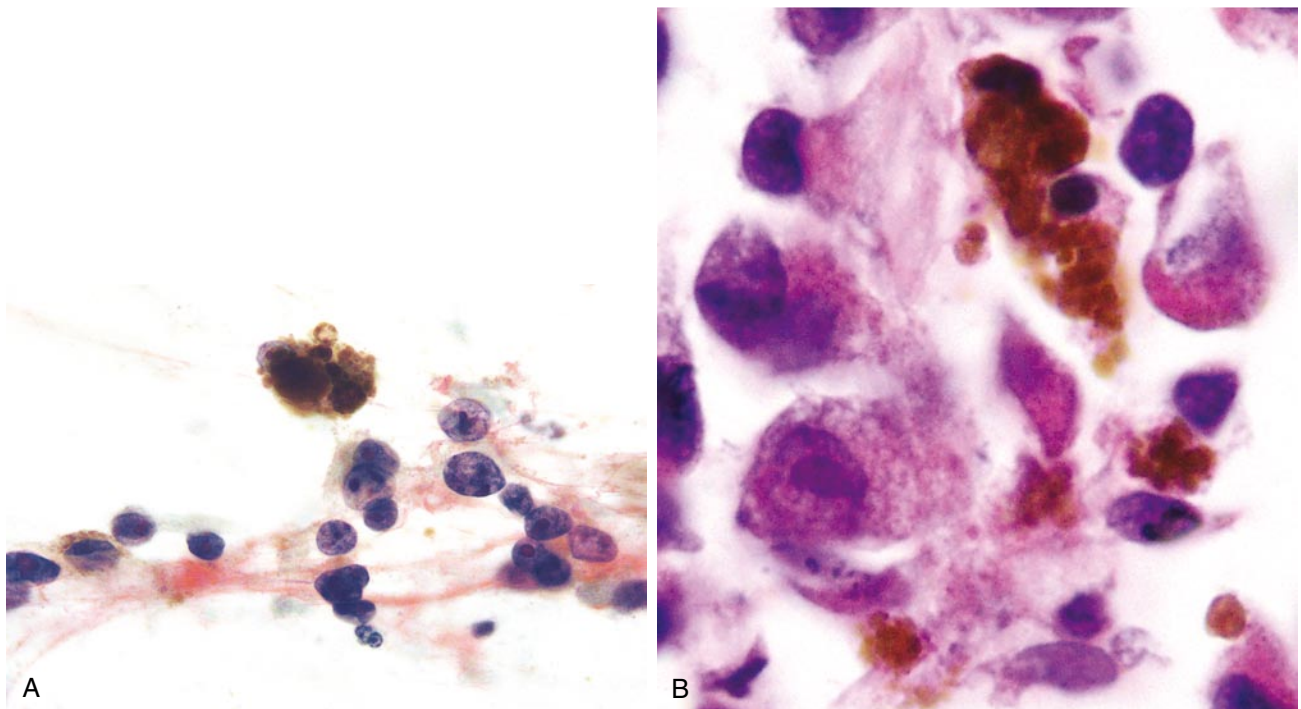


Figure 12.28 Metastatic melanoma. Although metastatic melanoma and hepatoma share many cytologic features, such as pigmented cells, prominent nucleoli, and intranuclear inclusions, they can be distinguished. A, B, Melanin tends to be more finely granular than bile, and more often is packed within cells. Melanoma cells are usually isolated rather than clustered and lack wrapping endothelial cells. The nucleoli of melanoma cells are usually prominent and solitary. (A, Papanicolaou stain, B, cell block, hematoxylin-eosin [H & E]).

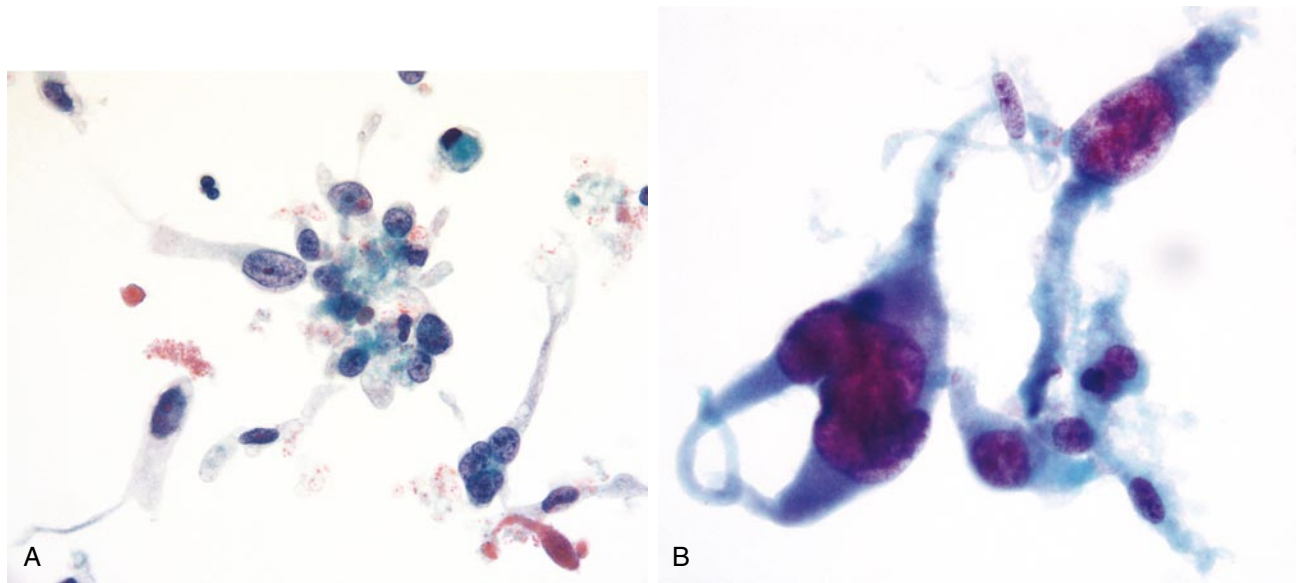


Figure 12.29 Metastatic sarcoma. The cells are predominantly spindled and can be quite pleomorphic. Although poorly differentiated hepatocellular carcinomas may contain bizarre spindled forms, the spindle cell component is usually not so prominent. A, Metastatic undifferentiated sarcoma (ThinPrep, Papanicolaou stain). B, Metastatic poorly differentiated leiomyosarcoma (ThinPrep, Papanicolaou stain).

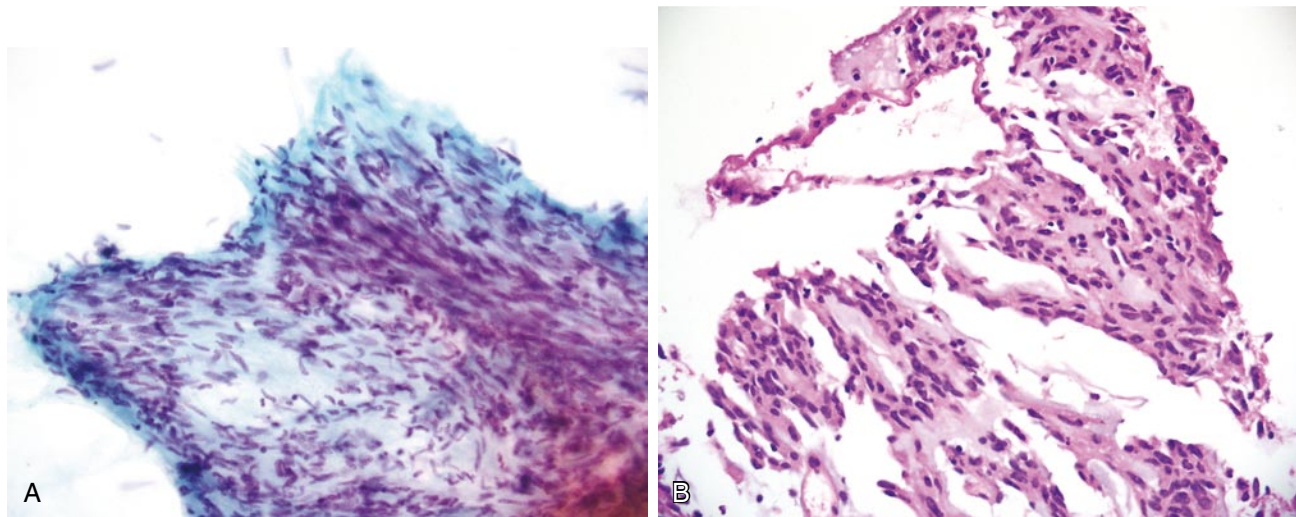


Figure 12.30 Metastatic gastrointestinal stromal tumor (GIST). A, There are cohesive groups of spindle-shaped cells. Nuclear atypia is minimal in this case (Papanicolaou stain). B, The spindle-cell proliferation is also apparent on cell block sections. The tumor cells were immunoreactive for CD117 (c-kit; not shown). (Hematoxylin-eosin [H & E] stain).

REFERENCES

1. Edoute Y, Tibon-Fisher O, Ben-Haim SA, Malberger E: Imaging-guided and nonimaging-guided fine needle aspiration of liver lesions: experience with 406 patients. *J Surg Oncol* 1991;48(4):246-251.
2. Borzio M, Borzio F, Macchi R, et al.: The evaluation of fine-needle procedures for the diagnosis of focal liver lesions in cirrhosis. *J Hepatol* 1994;20(1):117-121.
3. Edoute Y, Tibon-Fisher O, Ben Haim S, Malberger E: Ultrasonically guided fine-needle aspiration of liver lesions. *Am J Gastroenterol* 1992;87(9):1138-1141.
4. Ljubicic N, Bilic A, Lang N, Bakula B: Ultrasonically guided percutaneous fine needle aspiration biopsy of the hepatic and pancreatic focal lesions: Accuracy of cytology in the diagnosis of malignancy. *J R Soc Med* 1992;85(3):139-141.
5. Longchamps E, Patriarche C, Fabre M: Accuracy of cytology vs. microbiopsy for the diagnosis of well-differentiated hepatocellular carcinoma and macrorregenerative nodule. Definition of standardized criteria from a study of 100 cases. *Acta Cytol* 2000;44(4):515-523.
6. Luning M, Schroder K, Wolff H, et al.: Percutaneous biopsy of the liver. *Cardiovasc Intervent Radiol* 1991;14(1):40-42.
7. Bognel C, Rougier P, Leclerc J, et al.: Fine needle aspiration of the liver and pancreas with ultrasound guidance. *Acta Cytol* 1988;32(1):22-26.
8. Michielsen PP, Duysburgh IK, Francque SM, et al.: Ultrasonically guided fine needle puncture of focal liver

- lesions. Review and personal experience. *Acta Gastroenterol Belg* 1998;61(2):158-163.
9. Samaratunga H, Wright G: Value of fine needle aspiration biopsy cytology in the diagnosis of discrete hepatic lesions suspicious for malignancy. *Aust N Z J Surg* 1992;62(7):540-544.
 10. Santos G, Morini SR, Granero LC, et al.: Computed tomography-guided fine-needle aspiration biopsy. *Rev Paul Med* 1997;115(1):1343-1348.
 11. Sheikh M, Sawhney S, Dey P, et al.: Deep-seated thoracic and abdominal masses: Usefulness of ultrasound and computed tomography guidance in fine needle aspiration cytology diagnosis. *Australas Radiol* 2000;44(2):155-160.
 12. Zardawi IM: Fine needle aspiration cytology in a rural setting. *Acta Cytol* 1998;42(4):899-906.
 13. Fornari F, Civardi G, Cavanna L, et al.: Ultrasonically guided fine-needle aspiration biopsy: A highly diagnostic procedure for hepatic tumors. *Am J Gastroenterol* 1990;85(8):1009-1013.
 14. Fornari F, Filice C, Rapaccini GL, et al.: Small (< or = 3 cm) hepatic lesions. Results of sonographically guided fine-needle biopsy in 385 patients. *Dig Dis Sci* 1994;39(10):2267-2275.
 15. Kariniemi J, Blanco Sequeiros R, Ojala R, Tervonen O: MRI-guided abdominal biopsy in a 0.23-T open-configuration MRI system. *Eur Radiol* 2005;15(6):1256-1262.
 16. DeWitt J, LeBlanc J, McHenry L, et al.: Endoscopic ultrasound-guided fine needle aspiration cytology of solid liver lesions: A large single-center experience. *Am J Gastroenterol* 2003;98(9):1976-1981.
 17. Hollerbach S, Willert J, Topalidis T, et al.: Endoscopic ultrasound-guided fine-needle aspiration biopsy of liver lesions: histological and cytological assessment. *Endoscopy* 2003;35(9):743-749.
 18. TenBerge J, Hoffman BJ, Hawes RH, et al.: EUS-guided fine needle aspiration of the liver: Indications, yield, and safety based on an international survey of 167 cases. *Gastrointest Endosc* 2002;55(7):859-862.
 19. Guo Z, Kurtycz DF, Salem R, et al.: Radiologically guided percutaneous fine-needle aspiration biopsy of the liver: Retrospective study of 119 cases evaluating diagnostic effectiveness and clinical complications. *Diagn Cytopathol* 2002;26(5):283-289.
 20. Stewart CJ, Coldewey J, Stewart IS: Comparison of fine needle aspiration cytology and needle core biopsy in the diagnosis of radiologically detected abdominal lesions. *J Clin Pathol* 2002;55(2):93-97.
 21. Hajdu SI, D'Ambrosio FG, Fields V, Lightdale CJ: Aspiration and brush cytology of the liver. *Semin Diagn Pathol* 1986;3(3):227-238.
 22. Lin CC, Lin CJ, Hsu CW, et al.: Fine-needle aspiration cytology to distinguish dysplasia from hepatocellular carcinoma with different grades. *J Gastroenterol Hepatol* Epub May 27, 2007.
 23. Fritscher-Ravens A, Broering DC, Sriram PV, et al.: EUS-guided fine-needle aspiration cytodiagnosis of hilar cholangiocarcinoma: a case series. *Gastrointest Endosc* 2000;52(4):534-540.
 24. Nguyen P, Feng JC, Chang KJ: Endoscopic ultrasound (EUS) and EUS-guided fine-needle aspiration (FNA) of liver lesions. *Gastrointest Endosc* 1999;50(3):357-361.
 25. Lautenschlager I, Nashan B, Schlitt HJ, et al.: Different cellular patterns associated with hepatitis C virus reactivation, cytomegalovirus infection, and acute rejection in liver transplant patients monitored with transplant aspiration cytology. *Transplantation* 1994;58(12):1339-1345.
 26. Yussim A, Shapira Z, Nakache R, et al.: The utility of fine-needle aspiration biopsy in organ transplantation. *Transplant Proc* 1992;24(5):1880-1881.
 27. Hayry P, Lautenschlager I: Fine-needle aspiration biopsy in transplantation pathology. *Semin Diagn Pathol* 1992;9(3):232-237.
 28. Lautenschlager I, Hayry P: Laboratory monitoring of nonrenal allograft rejection. *Clin Lab Med* 1991;11(3):653-670.
 29. Kubota K, Ericzon BG, Reinholt FP: Comparison of fine-needle aspiration biopsy and histology in human liver transplants. *Transplantation* 1991;51(5):1010-1013.
 30. Schlitt HJ, Nashan B, Ringe B, et al.: Routine monitoring of liver grafts by transplant aspiration cytology: Clinical experience with 3000 TACs. *Transplant Proc* 1993;25(2):1970-1971.
 31. Gattuso P, Reddy VB, Kizilbash N, et al.: Role of fine-needle aspiration in the clinical management of solid organ transplant recipients: A review. *Cancer* 1999;87(5):286-294.
 32. Martinez-Noguera A, Donoso L, Coscojuela P: Fatal bleeding after fine-needle aspiration biopsy of a small hepatocarcinoma. *AJR Am J Roentgenol* 1991;156(5):1114-1115.
 33. Navarro F, Taourel P, Michel J, et al.: Diaphragmatic and subcutaneous seeding of hepatocellular carcinoma following fine-needle aspiration biopsy. *Liver* 1998;18(4):251-254.
 34. Postovsky S, Elhasid R, Ben Arush MW, et al.: Local dissemination of hepatocellular carcinoma in a child after fine-needle aspiration. *Med Pediatr Oncol* 2001;36(6):667-668.
 35. Nakamuta M, Tanabe Y, Ohashi M, et al.: Transabdominal seeding of hepatocellular carcinoma after fine-needle aspiration biopsy. *J Clin Ultrasound* 1993;21(8):551-556.
 36. Isobe H, Imari Y, Sakai H, et al.: Subcutaneous seeding of hepatocellular carcinoma following fine-needle aspiration biopsy. *J Clin Gastroenterol* 1993;17(4):350-352.
 37. Tung WC, Huang YJ, Leung SW, et al.: Incidence of needle tract seeding and responses of soft tissue metastasis by hepatocellular carcinoma postradiotherapy. *Liver Int* 2007;27(2):192-200.
 38. Saborido BP, Diaz JC, de Los Galanes SJ, et al.: Does preoperative fine needle aspiration-biopsy produce tumor recurrence in patients following liver transplantation for hepatocellular carcinoma? *Transplant Proc* 2005;37(9):3874-3877.
 39. Agarwal PK, Husain N, Singh BN: Cytologic findings in aspirated hydatid fluid. *Acta Cytol* 1989;33(5):652-654.
 40. Riska H, Friman C: Letter: Fatality after fine-needle aspiration biopsy of liver. *Br Med J* 1975;1(5956):517.
 41. Tobkes AL, Nord HJ: Liver biopsy: review of methodology and complications. *Dig Dis* 1995;13(5):267-274.
 42. Yamada N, Shinzawa H, Ukai K, et al.: Subcutaneous seeding of small hepatocellular carcinoma after fine needle aspiration biopsy. *J Gastroenterol Hepatol* 1993;8(2):195-198.
 43. Regev A, Reddy KR, Berho M, et al.: Large cystic lesions of the liver in adults: A 15-year experience in a tertiary center. *J Am Coll Surg* 2001;193(1):36-45.
 44. Bret PM, Fond A, Bretagnolle M, et al.: Percutaneous aspiration and drainage of hydatid cysts in the liver. *Radiology* 1988;168(3):617-620.
 45. Khuroo MS, Wani NA, Javid G, et al.: Percutaneous drainage compared with surgery for hepatic hydatid cysts. *N Engl J Med* 1997;337(13):881-887.
 46. Cohen MB, Haber MM, Holly EA, et al.: Cytologic criteria to distinguish hepatocellular carcinoma from nonneoplastic liver. *Am J Clin Pathol* 1991;95(2):125-130.
 47. Hill KA, Nayar R, DeFrias DV: Cytohistologic correlation of cirrhosis and hepatocellular carcinoma. Pitfall in diagnosis? *Acta Cytol* 2004;48(2):127-132.
 48. Yang GC, Yang GY, Tao LC: Distinguishing well-differentiated hepatocellular carcinoma from benign liver by the physical features of fine-needle aspirates. *Mod Pathol* 2004;17(7):798-802.
 49. Gonzalez F, Marks C: Hepatic tumors and oral contraceptives: surgical management. *J Surg Oncol* 1985;29(3):193-197.
 50. Ruschenburg I, Droese M: Fine needle aspiration cytology of focal nodular hyperplasia of the liver. *Acta Cytol* 1989;33(6):857-860.

51. Layfield LJ, Mooney EE, Dodd LG: Not by blood alone: diagnosis of hemangiomas by fine-needle aspiration. *Diagn Cytopathol* 1998;19(4):250-254.
52. Guy CD, Yuan S, Ballo MS: Spindle-cell lesions of the liver: diagnosis by fine-needle aspiration biopsy. *Diagn Cytopathol* 2001;25(2):94-100.
53. Sajima S, Kinoshita H, Okuda K, et al.: Angiomyolipoma of the liver—A case report and review of 48 cases reported in Japan. *Kurume Med J* 1999;46(2):127-131.
54. Xu AM, Zhang SH, Zheng JM, et al.: Pathological and molecular analysis of sporadic hepatic angiomyolipoma. *Hum Pathol* 2006;37(6):735-741.
55. Cha I, Cartwright D, Guis M, et al.: Angiomyolipoma of the liver in fine-needle aspiration biopsies: Its distinction from hepatocellular carcinoma. *Cancer* 1999;87(1):25-30.
56. Crapanzano JP: Fine-needle aspiration of renal angiomyolipoma: Cytological findings and diagnostic pitfalls in a series of five cases. *Diagn Cytopathol* 2005;32(1):53-57.
57. Pedio G, Landolt U, Zobeli L, Gut D: Fine needle aspiration of the liver. Significance of hepatocytic naked nuclei in the diagnosis of hepatocellular carcinoma. *Acta Cytol* 1988;32(4):437-442.
58. Bottles K, Cohen MB: An approach to fine-needle aspiration biopsy diagnosis of hepatic masses. *Diagn Cytopathol* 1991;7(2):204-210.
59. Sharifi S, Hayek J, Khettry U, Nasser I: Immunocytochemical staining of Kupffer and endothelial cells in fine needle aspiration cytology of hepatocellular carcinoma. *Acta Cytol* 2000;44(1):7-12.
60. Das DK: Cytodiagnosis of hepatocellular carcinoma in fine-needle aspirates of the liver: Its differentiation from reactive hepatocytes and metastatic adenocarcinoma. *Diagn Cytopathol* 1999;21(6):370-377.
61. de Boer WB, Segal A, Frost FA, Sterrett GF: Cytodiagnosis of well differentiated hepatocellular carcinoma: Can indeterminate diagnoses be reduced? *Cancer* 1999;87(5):270-277.
62. Wee A, Nilsson B, Chan-Wilde C, Yap I: Fine needle aspiration biopsy of hepatocellular carcinoma. Some unusual features. *Acta Cytol* 1991;35(6):661-670.
63. Kuo FY, Chen WJ, Lu SN, et al.: Fine needle aspiration cytodiagnosis of liver tumors. *Acta Cytol* 2004;48(2):142-148.
64. Pitman MB, Szyfelbein WM: Significance of endothelium in the fine-needle aspiration biopsy diagnosis of hepatocellular carcinoma. *Diagn Cytopathol* 1995;12(3):208-214.
65. Yang GC, Yang GY, Tao LC: Cytologic features and histologic correlations of microacinar and microtrabecular types of well-differentiated hepatocellular carcinoma in fine-needle aspiration biopsy. *Cancer* 2004;102(1):27-33.
66. Yu GH, Gustafson KS, Pan ST, Wetherington RW: Peripheral endothelial cells are not reliable in differentiating primary benign and malignant hepatocellular lesions in fine needle aspirates of the liver. *Cytopathology* 2002;13(3):145-151.
67. Sole M, Calvet X, Cuberes T, et al.: Value and limitations of cytologic criteria for the diagnosis of hepatocellular carcinoma by fine needle aspiration biopsy. *Acta Cytol* 1993;37(3):309-316.
68. Kong CS, Appenzeller M, Ferrell LD: Utility of CD34 reactivity in evaluating focal nodular hepatocellular lesions sampled by fine needle aspiration biopsy. *Acta Cytol* 2000;44(2):218-222.
69. de Boer WB, Segal A, Frost FA, Sterrett GF: Can CD34 discriminate between benign and malignant hepatocytic lesions in fine-needle aspirates and thin core biopsies? *Cancer* 2000;90(5):273-278.
70. Wee A: Diagnostic utility of immunohistochemistry in hepatocellular carcinoma, its variants and their mimics. *Appl Immunohistochem Mol Morphol* 2006;14(3):266-272.
71. Bergman S, Graeme-Cook F, Pitman MB: The usefulness of the reticulin stain in the differential diagnosis of liver nodules on fine-needle aspiration biopsy cell block preparations. *Mod Pathol* 1997;10(12):1258-1264.
72. Gagliano EF: Reticulin stain in the fine needle aspiration differential diagnosis of liver nodules. *Acta Cytol* 1995;39(3):596-598.
73. Libbrecht L, Severi T, Cassiman D, et al.: Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. *Am J Surg Pathol* 2006;30(11):1405-1411.
74. Senes G, Fanni D, Cois A, et al.: Intratumoral sampling variability in hepatocellular carcinoma: A case report. *World J Gastroenterol* 2007;13(29):4019-4021.
75. Llovet JM, Chen Y, Wurmbach E, et al.: A molecular signature to discriminate dysplastic nodules from early hepatocellular carcinoma in HCV cirrhosis. *Gastroenterology* 2006;131(6):1758-1767.
76. Wang XY, Degos F, Dubois S, et al.: Glypican-3 expression in hepatocellular tumors: Diagnostic value for preneoplastic lesions and hepatocellular carcinomas. *Hum Pathol* 2006;37(11):1435-1441.
77. Yamauchi N, Watanabe A, Hishinuma M, et al.: The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Mod Pathol* 2005;18(12):1591-1598.
78. Guzman G, Wu SJ, Kajdacsy-Balla A, Cotler SJ: Alpha-methylacyl-CoA racemase (AMACR/P504S) can distinguish hepatocellular carcinoma and dysplastic hepatocytes from benign nondysplastic hepatocytes. *Appl Immunohistochem Mol Morphol* 2006;14(4):411-416.
79. Went PT, Sauter G, Oberholzer M, Bubendorf L: Abundant expression of AMACR in many distinct tumour types. *Pathology* 2006;38(5):426-432.
80. Ojanguren I, Ariza A, Llatjos M, et al.: Proliferating cell nuclear antigen expression in normal, regenerative, and neoplastic liver: A fine-needle aspiration cytology and biopsy study. *Hum Pathol* 1993;24(8):905-908.
81. Deprez C, Vangansbeke D, Fastrez R, et al.: Nuclear DNA content, proliferation index, and nuclear size determination in normal and cirrhotic liver, and in benign and malignant primary and metastatic hepatic tumors. *Am J Clin Pathol* 1993;99(5):558-565.
82. Siddiqui MS, Soomro IN, Kayani N, et al.: Assessment of nucleolar organizer regions (NORs) in proliferative conditions of the liver. *Pathol Res Pract* 1999;195(6):421-426.
83. Zeppa P, Benincasa G, Troncone G, et al.: Retrospective evaluation of DNA ploidy of hepatocarcinoma on cytologic samples. *Diagn Cytopathol* 1998;19(5):323-329.
84. Zeppa P, Vetrani A, Palombini L, et al.: Evolution of DNA content in small and well-differentiated hepatocarcinoma. *Anal Quant Cytol Histol* 1993;15(1):12-22.
85. van Dekken H, Verhoef C, Wink J, et al.: Cell biological evaluation of liver cell carcinoma, dysplasia and adenoma by tissue micro-array analysis. *Acta Histochem* 2005;107(3):161-171.
86. Ojanguren I, Ariza A, Castella EM, et al.: p53 immunoreactivity in hepatocellular adenoma, focal nodular hyperplasia, cirrhosis and hepatocellular carcinoma. *Histopathology* 1995;26(1):63-68.
87. Ojanguren I, Castella E, Llatjos M, et al.: p53 immunoreaction in hepatocellular carcinoma and its relationship to etiologic factors. A fine needle aspiration study. *Acta Cytol* 1996;40(6):1148-1153.
88. Wee A, Nilsson B: Combined hepatocellular-cholangiocarcinoma. Diagnostic challenge in hepatic fine needle aspiration biopsy. *Acta Cytol* 1999;43(2):131-138.
89. Kilpatrick SE, Geisinger KR, Loggie BW, Hopkins MB 3rd: Cytomorphology of combined hepatocellular-cholangiocarcinoma in fine needle aspirates of the liver. A report of two cases. *Acta Cytol* 1993;37(6):943-947.

90. Gibbons D, de las Morenas A: Fine needle aspiration diagnosis of combined hepatocellular carcinoma and cholangiocarcinoma. A case report. *Acta Cytol* 1997;41 (4 Suppl):1269-1272.
91. Singh HK, Silverman JF, Geisinger KR: Fine-needle aspiration cytomorphology of clear-cell hepatocellular carcinoma. *Diagn Cytopathol* 1997;17(4):306-310.
92. Donat EE, Anderson V, Tao LC: Cytodiagnosis of clear cell hepatocellular carcinoma. A case report. *Acta Cytol* 1991;35(6):671-675.
93. Nayar R, Boursos E, DeFrias DV: Hyaline globules in renal cell carcinoma and hepatocellular carcinoma. A clue or a diagnostic pitfall on fine-needle aspiration? *Am J Clin Pathol* 2000;114(4):576-582.
94. Kung IT, Chan SK, Fung KH: Fine-needle aspiration in hepatocellular carcinoma. Combined cytologic and histologic approach. *Cancer* 1991;67(3):673-680.
95. Ma CK, Zarbo RJ, Frierson HF Jr, Lee MW: Comparative immunohistochemical study of primary and metastatic carcinomas of the liver. *Am J Clin Pathol* 1993;99(5):551-557.
96. Minervini MI, Demetris AJ, Lee RG, et al.: Utilization of hepatocyte-specific antibody in the immunocytochemical evaluation of liver tumors. *Mod Pathol* 1997;10(7):686-692.
97. Johnson DE, Powers CN, Rupp G, Frable WJ: Immunocytochemical staining of fine-needle aspiration biopsies of the liver as a diagnostic tool for hepatocellular carcinoma. *Mod Pathol* 1992;5(2):117-123.
98. Wolber RA, Greene CA, Dupuis BA: Polyclonal carcinoembryonic antigen staining in the cytologic differential diagnosis of primary and metastatic hepatic malignancies. *Acta Cytol* 1991;35(2):215-220.
99. Carrozza MJ, Calafati SA, Edmonds PR: Immunocytochemical localization of polyclonal carcinoembryonic antigen in hepatocellular carcinomas. *Acta Cytol* 1991;35(2):221-224.
100. Wee A, Nilsson B: pCEA canalicular immunostaining in fine needle aspiration biopsy diagnosis of hepatocellular carcinoma. *Acta Cytol* 1997;41(4):1147-1155.
101. Guindi M, Yazdi HM, Gilliatt MA: Fine needle aspiration biopsy of hepatocellular carcinoma. Value of immunocytochemical and ultrastructural studies. *Acta Cytol* 1994;38(3):385-391.
102. Christensen WN, Boitnott JK, Kuhajda FP: Immunoperoxidase staining as a diagnostic aid for hepatocellular carcinoma. *Mod Pathol* 1989;2(1):8-12.
103. Panicker JN, Shenoy KT, Augustine J: A cytological study of hepatitis B surface antigen localization using orcein staining in hepatocellular carcinoma. *Indian J Med Res* 1996;104:374-376.
104. Zimmerman RL, Burke MA, Young NA, et al.: Diagnostic value of hepatocyte paraffin 1 antibody to discriminate hepatocellular carcinoma from metastatic carcinoma in fine-needle aspiration biopsies of the liver. *Cancer* 2001;93(4):288-291.
105. Lugli A, Tornillo L, Mirlacher M, et al.: Hepatocyte paraffin 1 expression in human normal and neoplastic tissues: tissue microarray analysis on 3,940 tissue samples. *Am J Clin Pathol* 2004;122(5):721-727.
106. Gokden M, Shinde A: Recent immunohistochemical markers in the differential diagnosis of primary and metastatic carcinomas of the liver. *Diagn Cytopathol* 2005;33(3):166-172.
107. Wang L, Vuolo M, Suhrland MJ, Schlesinger K: HepPar1, MOC-31, pCEA, mCEA and CD10 for distinguishing hepatocellular carcinoma vs. metastatic adenocarcinoma in liver fine needle aspirates. *Acta Cytol* 2006;50(3):257-262.
108. Saad RS, Luckasevic TM, Noga CM, et al.: Diagnostic value of HepPar1, pCEA, CD10, and CD34 expression in separating hepatocellular carcinoma from metastatic carcinoma in fine-needle aspiration cytology. *Diagn Cytopathol* 2004;30(1):1-6.
109. Stroescu C, Herlea V, Dragnea A, Popescu I: The diagnostic value of cytokeratins and carcinoembryonic antigen immunostaining in differentiating hepatocellular carcinomas from intrahepatic cholangiocarcinomas. *J Gastrointest Liver Dis* 2006;15(1):9-14.
110. Durnez A, Verslype C, Nevens F, et al.: The clinicopathological and prognostic relevance of cytokeratin 7 and 19 expression in hepatocellular carcinoma. A possible progenitor cell origin. *Histopathology* 2006;49(2):138-151.
111. Lei JY, Bourne PA, diSant'Agnese PA, Huang J: Cytoplasmic staining of TTF-1 in the differential diagnosis of hepatocellular carcinoma vs cholangiocarcinoma and metastatic carcinoma of the liver. *Am J Clin Pathol* 2006;125(4):519-525.
112. Pan CC, Chen PC, Tsay SH, Chiang H: Cytoplasmic immunoreactivity for thyroid transcription factor-1 in hepatocellular carcinoma: a comparative immunohistochemical analysis of four commercial antibodies using a tissue array technique. *Am J Clin Pathol* 2004;121(3):343-349.
113. Wieczorek TJ, Pinkus JL, Glickman JN, Pinkus GS: Comparison of thyroid transcription factor-1 and hepatocyte antigen immunohistochemical analysis in the differential diagnosis of hepatocellular carcinoma, metastatic adenocarcinoma, renal cell carcinoma, and adrenal cortical carcinoma. *Am J Clin Pathol* 2002;118(6):911-921.
114. Pang Y, von Turkovich M, Wu H, et al.: The binding of thyroid transcription factor-1 and hepatocyte paraffin 1 to mitochondrial proteins in hepatocytes: A molecular and immunoelectron microscopic study. *Am J Clin Pathol* 2006;125(5):722-726.
115. Zimmerman RL, Logani S, Baloch Z: Evaluation of the CD34 and CD10 immunostains using a two-color staining protocol in liver fine-needle aspiration biopsies. *Diagn Cytopathol* 2005;32(2):88-91.
116. Wu HH, Tao LC, Cramer HM: Vimentin-positive spider-shaped Kupffer cells. A new clue to cytologic diagnosis of primary and metastatic hepatocellular carcinoma by fine-needle aspiration biopsy. *Am J Clin Pathol* 1996;106(4):517-521.
117. Onofre AS, Pomjanski N, Buckstegge B, Bocking A: Immunocytochemical diagnosis of hepatocellular carcinoma and identification of carcinomas of unknown primary metastatic to the liver on fine-needle aspiration cytologies. *Cancer* 2007;111(4):259-268.
118. Perez-Guillermo M, Masgrau NA, Garcia-Solano J, et al.: Cytologic aspect of fibrolamellar hepatocellular carcinoma in fine-needle aspirates. *Diagn Cytopathol* 1999;21(3):180-187.
119. Klein WM, Molmenti EP, Colombani PM, et al.: Primary liver carcinoma arising in people younger than 30 years. *Am J Clin Pathol* 2005;124(4):512-518.
120. Yeh MM: Immunohistochemical analysis in hepatocellular carcinoma: Does age matter? *Am J Clin Pathol* 2005;124(4):491-493.
121. Gornicka B, Ziarkiewicz-Wroblewska B, Wroblewski T, et al.: Carcinoma, a fibrolamellar variant—immunohistochemical analysis of 4 cases. *Hepatogastroenterology* 2005;52(62):519-523.
122. Thuluvath PJ, Rai R, Venbrux AC, Yeo CJ: Cholangiocarcinoma: A review. *Gastroenterologist* 1997;5(4):306-315.
123. Desa LA, Akosa AB, Lazzara S, et al.: Cytodiagnosis in the management of extrahepatic biliary stricture. *Gut* 1991;32(10):1188-1191.
124. Singh P, Erickson RA, Mukhopadhyay P, et al.: EUS for detection of the hepatocellular carcinoma: Results of a prospective study. *Gastrointest Endosc* 2007;66(2):265-273.
125. DeWitt J, Misra VL, Leblanc JK, et al.: EUS-guided FNA of proximal biliary strictures after negative ERCP brush cytology results. *Gastrointest Endosc* 2006;64(3):325-333.
126. Chang KJ: State of the art lecture: endoscopic ultrasound (EUS) and FNA in pancreatico-biliary tumors. *Endoscopy* 2006;38 Suppl 1:S56-S60.

127. Meara RS, Jhala D, Eloubeidi MA, et al.: Endoscopic ultrasound-guided FNA biopsy of bile duct and gallbladder: Analysis of 53 cases. *Cytopathology* 2006;17(1):42-49.
128. Eloubeidi MA, Chen VK, Jhala NC, et al.: Endoscopic ultrasound-guided fine needle aspiration biopsy of suspected cholangiocarcinoma. *Clin Gastroenterol Hepatol* 2004;2(3):209-213.
129. Mizukami Y, Ohta H, Arisato S, et al.: Case report: Mucinous cholangiocarcinoma featuring a multicystic appearance and periportal collar in imaging. *J Gastroenterol Hepatol* 1999;14(12):1223-1226.
130. Sampatanukul P, Leong AS, Kosolbhand P, Tangkijvanich P: Proliferating ductules are a diagnostic discriminator for intrahepatic cholangiocarcinoma in FNA biopsies. *Diagn Cytopathol* 2000;22(6):359-363.
131. Sola Perez J, Perez-Guillermo M, Bas Bernal AB, Mercader JM: Hepatoblastoma. An attempt to apply histologic classification to aspirates obtained by fine needle aspiration cytology. *Acta Cytol* 1994;38(2):175-182.
132. Kaw YT, Hansen K: Fine needle aspiration cytology of undifferentiated small cell ("anaplastic") hepatoblastoma. A case report. *Acta Cytol* 1993;37(2):216-220.
133. Philipose TR, Naik R, Rai S: Cytologic diagnosis of small cell anaplastic hepatoblastoma: A case report. *Acta Cytol* 2006;50(2):205-7.
134. Hertzanu Y, Peiser J, Zirkin H. Massive bleeding after fine needle aspiration of liver angiosarcoma. *Gastrointest Radiol* 1990;15(1):43-46.
135. Wakely PE Jr, Frable WJ, Kneisl JS: Aspiration cytopathology of epithelioid angiosarcoma. *Cancer* 2000;90(4):245-251.
136. Boucher LD, Swanson PE, Stanley MW, et al.: Cytology of angiosarcoma. Findings in fourteen fine-needle aspiration biopsy specimens and one pleural fluid specimen. *Am J Clin Pathol* 2000;114(2):210-219.
137. Cho NH, Lee KG, Jeong MG: Cytologic evaluation of primary malignant vascular tumors of the liver. One case each of angiosarcoma and epithelioid hemangioendothelioma. *Acta Cytol* 1997;41(5):1468-1476.
138. Wee A, Nilsson B: Fine needle aspiration biopsy of hepatic leiomyosarcoma. An unusual epithelioid variant posing a potential diagnostic pitfall in a hepatocellular carcinoma-prevalent population. *Acta Cytol* 1997;41(3):737-743.
139. Smith MB, Silverman JF, Raab SS, et al.: Fine-needle aspiration cytology of hepatic leiomyosarcoma. *Diagn Cytopathol* 1994;11(4):321-327.
140. Kiyozuka Y, Koyama H, Nakata M, et al.: Diagnostic cytopathology in type II angiosarcoma of the breast: a case report. *Acta Cytol* 2005;49(5):560-566.
141. Bhalla R, Nassar A: Cardiac angiosarcoma: Report of a case diagnosed by echocardiographic-guided fine-needle aspiration. *Diagn Cytopathol* 2007;35(3):164-166.
142. Gambacorta M, Bonacina E: Epithelioid hemangioendothelioma: Report of a case diagnosed by fine-needle aspiration. *Diagn Cytopathol* 1989;5(2):207-210.
143. Tong GX, Hamele-Bena D, Borczuk A, et al.: Fine needle aspiration biopsy of epithelioid hemangioendothelioma of the oral cavity: Report of one case and review of literature. *Diagn Cytopathol* 2006;34(3):218-223.
144. Hertz G, Reddy VB, Green L, et al.: Fine-needle aspiration biopsy of the liver: A multicenter study of 602 radiologically guided FNA. *Diagn Cytopathol* 2000;23(5):326-328.
145. Gupta RK, Naran S, Lallu S, Fauck R: Fine needle aspiration diagnosis of neuroendocrine tumors in the liver. *Pathology* 2000;32(1):16-20.
146. Rappaport KM, DiGiuseppe JA, Busseniers AE: Primary hepatic lymphoma: Report of two cases diagnosed by fine-needle aspiration. *Diagn Cytopathol* 1995;13(2):142-145.

Pancreas and Biliary Tree

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INDICATIONS

The principal indications for cytologic evaluation of the pancreas and bile ducts are a suspicious pancreatic mass and a duct stricture. Imaging studies contribute useful information on the location, distribution (solitary, multiple, or diffuse), and nature (cystic versus solid) of a mass or stricture, but, ultimately, a tissue diagnosis is necessary to guide clinical management.

Cytologic sampling methods include fine-needle aspiration (FNA) and duct brushings. FNA is effective for many pancreatic lesions. It can confirm or exclude malignancy in a minimally invasive manner, thereby preventing unnecessary surgery in patients with inoperable tumors and permitting neoadjuvant radiation and chemotherapy in those with potentially resectable malignancies.^{1,2} FNA is particularly well-suited for the diagnosis of a variety of solid tumors, including ductal adenocarcinoma, pancreatic endocrine neoplasms (PENs), acinar cell carcinoma (ACC), solid-pseudopapillary neoplasm (SPN), lymphoma, and metastatic neoplasms. Cystic pancreatic lesions can also be evaluated by FNA, but they present unique challenges that are discussed later in the chapter.

Duct brushings are generally used when there is a stricture without a clearly identifiable mass.

SAMPLING TECHNIQUES

An FNA of the pancreas can be performed percutaneously (by a radiologist) or endoscopically (by a gastro-

enterologist). To guide a *percutaneous pancreatic FNA*, two different imaging techniques are commonly used. Transabdominal ultrasound is a portable, rapid method that permits identification of the needle tip in real time. It requires no radiation, but bowel gas and obesity may hinder visualization of the lesion.¹ Computed tomography (CT) provides better resolution than ultrasound and permits visualization despite obesity or bowel gas, but because this method does not permit real-time visualization of the needle tip, it can be more time consuming and expensive.³

Percutaneous needle placement techniques vary depending on the imaging modality (CT versus ultrasound), the trajectory of the needle, and the location of the lesion. The tandem technique involves placing a guide needle to serve as a reference point for the second (biopsy needle) and is most useful in CT-guided FNA in which real-time visualization of needle insertion is impossible. The coaxial technique involves inserting a larger caliber needle to localize the lesion. A smaller caliber needle is then inserted through the larger needle to sample the lesion. This method permits multiple sampling attempts without the increased risk to local structures created by repeated needle passes.

Over the past 10 years, *endoscopic ultrasound-guided (EUS) FNA* has emerged as an alternative to percutaneous FNA.⁴⁻⁹ Its advantages include real-time visualization of the needle tip, better visualization of small lesions that can be missed by CT or transabdominal ultrasound, and identification of local metastases or invasion of local structures.^{2,4-6,10,11} This permits simultaneous diagnosis

and staging and aids in planning patient management.¹² In many medical centers, EUS-guided FNA has become the primary modality for obtaining an FNA sample from a pancreatic mass.^{5,11}

Suspicious pancreatic and extra-pancreatic biliary lesions can also be sampled by *brushing the pancreatic and common bile ducts*, particularly useful when there is a duct stricture without a discernible mass. Because pancreatic ductal adenocarcinoma and other pancreatic malignancies frequently invade the pancreatic or common bile ducts, this sampling method is highly effective. Brushings are also useful for diagnosing some cholangiocarcinomas¹³ and even a rare, accessible hepatocellular carcinoma (HCC).¹³ Brushings are usually performed endoscopically, sometimes in conjunction with endoscopic retrograde cholangiopancreatography (ERCP); they can also be obtained during percutaneous transhepatic cholangiography (PTC). Complications are minor and include cholangitis and mild pancreatitis.¹⁴ The sensitivity of brushings ranges from 44% to 72%, and specificity approaches 100%.¹³⁻¹⁹ Sensitivity is improved by brushing both pancreatic and common bile ducts.¹⁴

ACCURACY AND COMPLICATIONS

The sensitivity of FNA for detecting pancreatic malignancy ranges from 86% to 98% in samples obtained percutaneously²⁰⁻²³ and from 75% to 94% by EUS-guided FNA.^{7,8,12,24-29} Specificity, for both percutaneous and EUS-guided FNA, approaches 100%.^{12,21,24} The last decade has seen improvements both in the sampling and cytologic interpretation of pancreatic masses. Criteria for distinguishing pancreatic adenocarcinomas, particularly the well-differentiated tumors, from benign reactive processes have been refined.^{23,30,31} Some authors have found that rapid, on-site adequacy evaluation by a cytologist in the radiology or endoscopy suite improves diagnostic accuracy.³²⁻³⁹

Sampling or interpretation errors can contribute to the occasional false-negative diagnosis.^{17,23} Sampling error is associated with small lesion size, a difficult-to-target anatomic location, extensive fibrosis, and excessive bleeding. False-negatives because of an interpretation error most commonly result from misinterpreting a well-differentiated ductal adenocarcinoma as benign ductal cells. Less-than-optimal sample preparation can hamper interpretation and result in a false-negative diagnosis. False-positive diagnoses are less common and typically result from overinterpretation of reactive atypia in the setting of pancreatitis.

There are few contraindications to percutaneous FNA of the pancreas. These include an uncorrectable bleeding disorder and lack of a safe access route.⁴⁰ EUS-guided FNA has an additional contraindication, upper gastrointestinal (GI) obstruction, which prevents

the echoendoscope from reaching the desired location.⁴¹ The rate of minor complications (vasovagal reaction, pain, and small hemorrhage) with percutaneous FNA of the pancreas is 2.2%. EUS-guided FNA of the pancreas has a similar rate of minor complications (1% to 5%).^{12,24,27,28} The most common major complication is acute pancreatitis, with an incidence of 1% to 3%; death resulting from FNA-induced pancreatitis is rare.^{42,43} Other, rare major complications include pancreatic duct leaks, massive hemorrhage, and septic shock.⁴² A complication specific to EUS-guided FNA is perforation resulting from malignant luminal stenosis. Needle tract seeding by tumor cells following FNA of pancreatic malignancy is exceedingly rare (.006%).^{43,44} The reported cases of needle tract seeding may be attributable to numerous needle passes and intermediate-sized needles (19 or 20 gauge).^{45,46} Needle tract seeding has not been reported as a complication of EUS-guided FNA.³²

SAMPLE PREPARATION AND REPORTING TECHNOLOGY

Pertinent clinical and radiologic information are helpful for accurate interpretation of the specimen. The requisition form (or an easily accessible electronic medical record) should document the size and location of the lesion and the method used to obtain the specimen (pancreatic or common bile duct brushing, percutaneous or EUS-guided FNA). Knowing whether the lesion is solid, cystic, or necrotic and whether it is solitary, multifocal, unilocular, or multilocular can be valuable. Similarly, clinical evidence or history of pancreatitis, the use of stents to relieve obstruction, and a history of a prior malignancy of any type can help inform the cytologic interpretation.

The value of good sample preparation cannot be overemphasized. One common method is direct smear preparation at the time of aspiration or brushing. This works well if a cytologist trained in smearing techniques is present at the procedure. Commonly, some slides are air-dried and stained with a Romanowsky stain. Because of the availability of rapid Romanowsky-type stains (e.g., Diff-Quik®, Hemacolor®) that take less than 30 seconds to complete, these are often used for immediate evaluation of specimen adequacy, but other rapid staining methods (e.g., toluidine blue) are available. Smears can also be alcohol-fixed and stained with the Papanicolaou stain.⁴⁷ Instead of (or in addition to) smears, the brushing or FNA can be deposited into an alcohol-based preservative solution for liquid-based preparations (ThinPrep®, SurePath®), cytocentrifuge preparations, or cell blocks. Allocation of material for cell block preparation can be essential because cell blocks are ideal substrates for immunohistochemistry, which is a useful

method for distinguishing some pancreatic look-alikes (ACC, PEN, and SPN). At the time of specimen collection, additional consideration should be given to the need for ancillary studies, like microbiologic cultures, electron microscopy, or flow cytometric analysis, for which a dedicated needle pass may be needed. When aspirating a cystic lesion, allocation of a portion of fresh cyst fluid for the analysis of tumor markers, pancreatic enzymes, viscosity, and molecular markers can be quite helpful. Molecular analysis for *K-ras* and tumor suppressor gene-linked microsatellite marker allelic loss has emerged as a potential adjunct in the evaluation of pancreatic cysts.⁴⁸⁻⁵⁰ Pancreatic cyst fluid analysis may not be available in all laboratories. Information regarding appropriate storage and transfer of cyst fluid should be obtained from the affiliated or referral laboratory.^{41,51}

There is no universal standard for reporting pancreatic cytology results, but the following modification of conventional cytologic nomenclature can be a useful framework:

- negative for malignant cells
- neoplastic cells present
- atypical cells present
- suspicious for malignancy
- positive for malignant cells
- nondiagnostic specimen

A *negative* diagnosis implies a benign process like chronic pancreatitis, but the possibility that a malignant neoplasm was not sampled cannot be entirely excluded. Because of the possibility of sampling error, an explanatory note with every *negative* interpretation (e.g., “NOTE: Appropriate follow-up is recommended to ensure that the sample is representative of the underlying lesion.”) can be helpful. An *atypical* diagnosis reflects a mild degree of cytologic atypia, often in the setting of inflammation, prior duct instrumentation, or sparse cellularity. This interpretation implies a low suspicion of malignancy. *Suspicious for malignancy* implies a strong concern, but the cytologic features are quantitatively or qualitatively insufficient for a definitive diagnosis of malignancy. The *nondiagnostic* category (also called *unsatisfactory* or *insufficient*) is used when representative lesional tissue was not obtained (e.g., a virtually acellular specimen) or the cells are obscured beyond interpretation. This interpretation is often accompanied by an explanation (i.e., obscuring inflammation or blood, air-drying artifact, insufficient cellularity) and an explanatory note suggesting that repeat sampling be considered. A nondiagnostic interpretation as a result of the lack of epithelial cells is not infrequent with cystic lesions.

When cytologic findings are consistent with those of a neoplasm, but the distinction between a benign and malignant neoplasm is not possible, the category *neoplastic cells present* is appropriate. For example, this categorization applies when the principal cytologic finding

is mucinous epithelium without atypia, and imaging findings are consistent with either a mucinous cystic neoplasm (MCN) or an intraductal papillary mucinous neoplasm (IPMN). Depending on which scenario applies, “consistent with an MCN” or “consistent with an IPMN” can be used to modify the primary diagnostic heading. Because these tumors are often morphologically heterogeneous, precise classification requires extensive histologic sampling. Thus, cytologic samples showing only benign mucinous epithelium may not be representative of the entire neoplasm, and a negative interpretation might be misleading.

Neoplastic cells present is also an appropriate category for SPNs and PENs. SPNs are considered a low-grade malignancy,⁵² but most behave in a benign fashion, and the malignant counterpart (solid pseudopapillary carcinoma) is defined by histologic criteria of invasion (perineural, vascular, adjacent structures) that cannot be recognized by FNA.⁵³ Similarly, the metastatic potential of PENs cannot be accurately predicted from cytomorphic features.

CYST FLUID ANALYSIS

Cystic lesions of the pancreas offer special challenges to FNA. Because cyst lining cells (if any) may not be adequately sampled, pancreatic cysts can be impossible to classify precisely by cellular examination alone. Contemporary pancreatic cyst FNA evaluation relies on a multidisciplinary approach that combines chemistry and molecular diagnostics with cytologic evaluation.

When evaluating a cystic lesion of the pancreas, the viscosity, pancreatic enzyme levels, and tumor marker expression in the fluid can all aid in refining the differential diagnosis (Table 13.1). Nonmucinous cyst fluid

TABLE 13.1 PANCREATIC CYST FLUID CYOLOGY

Enzymes	Pseudocyst or Non-neoplastic Cyst	Neoplastic Cyst
amylase	high	low
lipase	high	low
leukocyte esterase	high	low
Viscosity	Nonmucinous Cyst	Mucinous Cyst
	viscosity < serum	viscosity > serum
Tumor Markers	Nonmucinous Cyst	Mucinous Cyst
CEA	not elevated	elevated
CA72-4	not elevated	elevated
CA19-9	not elevated	elevated
CA125	not elevated	elevated
CA15-3	not elevated	elevated

CA, cancer antigen; CEA, carcinoembryonic antigen.

has a lower relative viscosity compared to serum, whereas mucinous cysts consistently have a higher relative viscosity.⁵⁴ In the distinction between pseudocysts and neoplastic cysts, three enzyme tests have demonstrated use. Fluid amylase, lipase, and leukocyte esterase levels tend to be high in pseudocysts and low in neoplastic cysts.⁵⁴⁻⁵⁶

A variety of tumor markers, such as carcinoembryonic antigen (CEA),⁵⁴⁻⁵⁶ cancer antigen 125 (CA125),⁵⁴ CA72-4,⁵¹ CA19-9,^{57,58} and CA15-3,⁵⁷ have been studied for their value in discriminating among the major types of cystic neoplasms, particularly between mucinous and nonmucinous cysts. Of these, the CEA level is the most accurate.^{57,59} Cyst fluid CEA <5 ng/mL is highly characteristic of pseudocysts and serous cystadenomas, whereas values >800 ng/mL are highly predictive of mucinous neoplasms, with a specificity of 98%. The sensitivity of such an elevated CEA level for detecting mucinous neoplasms, however, is <50%.⁵⁸ Brugge and colleagues showed an accuracy of 79% with a much lower cut-off value (192 ng/mL) for the diagnosis of a mucinous neoplasm.^{57,59} Unfortunately, a standard cutoff value for cyst fluid CEA has not been established, and the values used are highly variable among institutions.

Although tumor marker levels tend to be higher in malignant cystic neoplasms (both ductal adenocarcinoma with cystic degeneration and mucinous cystadenocarcinoma), the distinction between benign and malignant mucinous cysts cannot be made on the results of this test alone.⁵⁷ More recently, molecular studies of cyst fluid DNA have revealed K-ras, tumor suppressor gene mutations, and telomerase activity in neoplastic mucinous cysts.^{48,49,60}

NORMAL PANCREAS AND BILE DUCT

Brushings and FNAs sometimes yield only normal cells. It is important to recognize them as normal elements of the pancreas and not mistake them as neoplastic.

CYTOMORPHOLOGY OF BENIGN PANCREATIC ACINAR CELLS:

- acinar arrangement or isolated cells
- eccentrically placed, round nucleus
- evenly distributed, finely granular chromatin
- inconspicuous nucleolus
- abundant granular cytoplasm
- indistinct cell borders

Acinar cells, the digestive enzyme-producing cells of the pancreas, are sometimes obtained by FNA (but not duct brushings) and are normally arranged in a small rounded group (acinus) that has a small lumen.

Occasional isolated cells are also present. Acinar cells have abundant granular cytoplasm, indistinct cell borders, and an eccentrically placed round nucleus with uniformly distributed, finely textured chromatin, and an inconspicuous nucleolus (Fig. 13.1).

CYTOMORPHOLOGY OF BENIGN PANCREATIC AND BILIARY DUCTAL CELLS:

- flat, cohesive sheets (few isolated cells)
- even nuclear spacing within sheets
- round to oval nucleus
- evenly distributed, finely granular chromatin
- inconspicuous nucleolus
- well-defined cytoplasmic boundaries

Pancreatic duct and biliary duct cells, seen on brushings and FNAs, are morphologically similar, cuboidal to columnar cells. They are arranged in flat, honeycomb-like sheets with distinct cell borders and evenly spaced, round-to-oval, uniform nuclei (Fig. 13.2A, B). With liquid-based preparations, some nuclei appear crenated, a common artifact that is easily recognized with experience. Nucleoli are inconspicuous. Goblet cells may be present in samples from the main pancreatic duct. Normal islet cells are rarely identified because of their relative paucity and fragility.

The pancreas is nestled in the retroperitoneum, with its head surrounded by the second portion of the duodenum. It is not far from the liver, transverse colon, stomach, spleen, and kidneys. It is not unusual, therefore, to find normal elements from one or more of these organs in a pancreatic FNA. Mesothelial cells, hepatocytes, renal tubular cells, and gastrointestinal (GI) cells need to be distinguished from the cells of an epithelial neoplasm of the pancreas. This pitfall is discussed in greater depth in the text that follows.

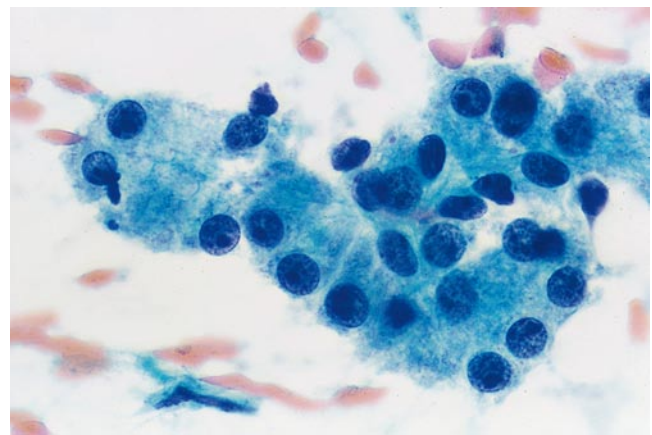


Figure 13.1 Normal pancreatic acinar cells. The acini are slightly disrupted and the lumen is not visible, but the characteristic abundant granular cytoplasm and eccentrically placed nucleus is apparent (Papanicolaou stain).

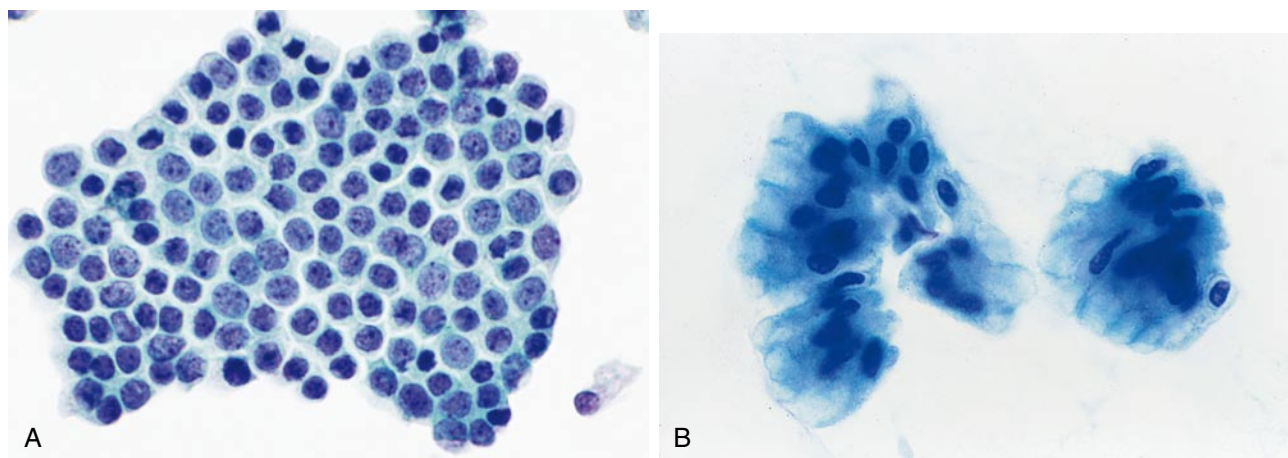


Figure 13.2 Normal pancreatic ductal cells. A, Normal ductal cells in FNAs and brushings are often arranged in large, cohesive sheets with evenly spaced nuclei that impart a honeycomb-like appearance (Papanicolaou stain). B, Some columnar ductal cells appear as palisading cells with basally located nuclei (Papanicolaou stain).

PANCREATITIS AND REACTIVE CHANGES

Acute pancreatitis results from the enzymatic autodestruction of pancreatic parenchyma and the accompanying acute inflammatory response. It is most commonly associated with biliary stones and alcoholism. The diagnosis of acute pancreatitis is nearly always based on clinical findings coupled with laboratory evidence of an elevated white blood cell count and elevated plasma amylase and lipase levels. The radiologic appearance is seldom suggestive of a mass lesion. For these reasons, acute pancreatitis is seldom aspirated. FNAs are composed of a background of necrotic debris upon which is superimposed degenerating cells, foamy histiocytes, fat necrosis, calcifications, and acute inflammation.

By contrast, *chronic pancreatitis* is difficult to distinguish radiologically from a neoplastic process. The cytologic appearance varies depending on the duration of the inflammation. The early stage is highly cellular and comprised of a mixed lymphohistiocytic infiltrate. The late stage is sparsely cellular and shows a relative predominance of islet cells resulting from acinar atrophy and extensive fibrosis of the pancreatic parenchyma.

In inflammatory conditions like acute and chronic pancreatitis, reactive ductal epithelium can demonstrate atypia (Fig. 13.3A, B), which, at its worst, is difficult to distinguish from that of a ductal adenocarcinoma. Mitoses, prominent nucleoli, and nuclear enlargement are present in reactive conditions and ductal adenocarcinoma. Reactive changes, sometime marked, can also be seen in bile duct brushings from patients with *primary sclerosing cholangitis*.^{61,62}

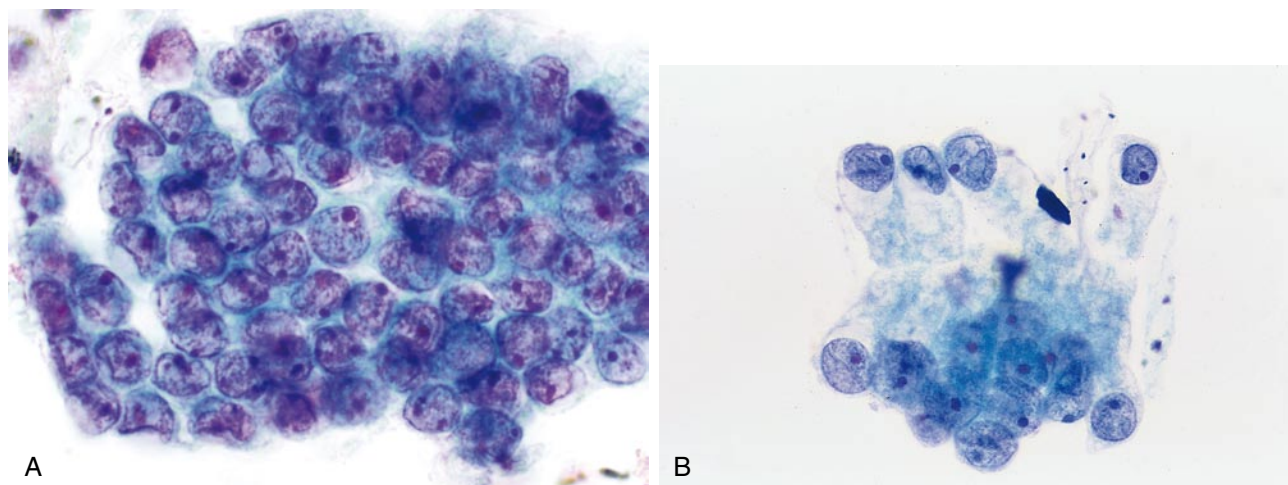


Figure 13.3 Reactive atypia of ductal epithelium (chronic pancreatitis). A, Note the nuclear enlargement and overlapping, and prominent nucleoli (Papanicolaou stain). B, The nuclei are basally located, with smooth, round contours and evenly distributed chromatin (Papanicolaou stain).

CYTOMORPHOLOGY OF REACTIVE DUCTAL ATYPIA:

- low cellularity
- flat, cohesive sheets with evenly spaced nuclei
- absent or only rare isolated atypical cells
- enlarged cells
- round to oval nucleus
- prominent nucleolus
- occasional mitoses
- abundant cytoplasm

The large cells, prominent nucleoli, and mild hyperchromasia raise the possibility of malignancy. But when cellularity is sparse, when isolated atypical cells are few or absent, and when the nuclear-to-cytoplasmic ratio is low, it is likely that such cells represent a reactive ductal cell atypia. In this setting, one of the equivocal interpretations (atypical cells present or suspicious for malignancy) may be appropriate, the precise choice depending on the degree of atypia and attendant level of suspicion. Preliminary data suggest that immunohistochemical studies for SMAD4 and p53 can be helpful in this distinction.⁶³ Most pancreatic adenocarcinomas show loss of SMAD4 and accumulation of p53, which are not seen in chronic pancreatitis.

PSEUDOCYST AND OTHER NON-NEOPLASTIC CYSTS

Pancreatic pseudocysts occur in the setting of acute pancreatitis and result from autodigestion of the pancreatic parenchyma. They account for the majority (75%) of pancreatic cysts.⁵² Pseudocysts account for the majority of cysts incorrectly diagnosed as neoplastic pancreatic cysts.⁶⁴ For this reason, the diagnosis of pseudocyst on cytology should be considered one of exclusion.

A pseudocyst, by definition, lacks an epithelial lining and is composed instead of an inflammatory fibrous capsule enclosing a region of necrosis. FNA yields either thin and cloudy or dark and turbid fluid. The specimen is composed principally of granular debris, macrophages (some of which contain hemosiderin), and bile. Aspirates may also contain the normal components of the adjacent pancreas (ductal epithelium, islet cells, and acinar cells), fibroblasts from the fibrous capsule, mesothelial cells, and gastric or duodenal epithelium, any of which might be mistaken for epithelial cyst lining cells.³

The differential diagnosis includes one of the neoplastic mucinous cystic lesions of the pancreas. This is an especially difficult distinction with EUS-guided specimens, which are almost always contaminated either by benign gastric or duodenal epithelium, depending on the trajectory of the needle. A neoplastic mucinous cyst should be considered if

the patient has no history or signs of pancreatitis. Chemical fluid analysis for CEA and amylase are especially helpful in this distinction. Amylase is virtually always elevated in pseudocysts. Amylase levels can be high in intraductal papillary mucinous tumors and even some MCNs, but a low amylase level essentially excludes a pseudocyst. An elevated CEA level is typical of mucinous neoplasms.⁵⁷

Other non-neoplastic cysts include retention cysts, cysts secondary to bacterial or parasitic infection, lymphoepithelial cysts,⁶⁵ and congenital cysts. These are extremely rare, and the diagnosis requires correlation with clinical, radiologic, and microbiologic findings.

DUCTAL ADENOCARCINOMA

Ductal adenocarcinoma is the most common tumor of the pancreas, comprising 85% to 90% of all pancreatic neoplasms.⁵³ Smoking is a well-established risk factor. Ductal adenocarcinoma occurs predominantly in individuals ages 60 to 80 and is found most commonly in the head of the pancreas (60% to 70%). Presenting symptoms include abdominal pain, jaundice, pruritus, and unexplained weight loss. Histologically, most ductal adenocarcinomas range from well-differentiated to moderately differentiated tumors, consisting of large, medium-sized, or small malignant ducts that infiltrate a fibrous stroma. Tubular, cribriform, and micropapillary architecture is common. The cells of well-differentiated tumors are large and columnar, often with abundant pale or granular cytoplasm. Nuclei show some loss of polarity and nucleoli are more prominent than in normal ductal cells, but there is little mitotic activity. Moderately differentiated tumors show greater variation in cell size, greater loss of polarity, larger nucleoli, and mitoses are more frequent. The uncommon poorly differentiated ductal adenocarcinomas show marked pleomorphism and numerous mitotic figures.

Cholangiocarcinomas are morphologically similar to pancreatic ductal adenocarcinomas.

CYTOMORPHOLOGY OF DUCTAL ADENOCARCINOMA:

- moderate to high cellularity
- crowded sheets of disordered ductal cells ("drunken honeycomb")
- isolated malignant cells
- irregular nuclear contours (notches, grooves, convolutions)
- nuclear enlargement (particularly marked anisonucleosis within a single sheet)
- irregular chromatin distribution (clumping and clearing)
- mitoses
- scant or abundant mucinous cytoplasm

Because ductal adenocarcinomas invade the pancreatic and common bile ducts, these tumors can be sampled by duct brushings and FNA. Cytologic features mirror the histologic findings. Because most ductal adenocarcinomas range from well differentiated to moderately differentiated, they often bear a strong resemblance to normal ductal cells. Like normal ductal cells, the cells of a well or moderately differentiated ductal adenocarcinoma are arranged in flat sheets. But a number of features help in the distinction.^{23,66-68} Ductal adenocarcinoma cells are larger than normal ductal cells and have distinct nucleoli. The loss of polarity typical of ductal adenocarcinoma cells is manifested cyto-

logically by crowding and uneven spacing that is quite different from the orderly, honeycomb-like arrangement of benign ductal cells (Fig. 13-4A). This disordered appearance has been termed a *drunken honeycomb*.⁶⁹ Marked nuclear size variation (anisonucleosis) is characteristic of ductal adenocarcinomas and often one of the first clues to the diagnosis (Fig. 13-4B). Nuclear contour irregularities and irregular chromatin distribution are also seen²³ (Fig. 13-4C). Finding frequent isolated atypical cells is also helpful because they are uncommon in benign, reactive conditions.^{23,67} Necrosis is seen in only one half of cases.²³ Mitoses are rare in well-differentiated ductal adenocarcinomas, more frequent in moderately

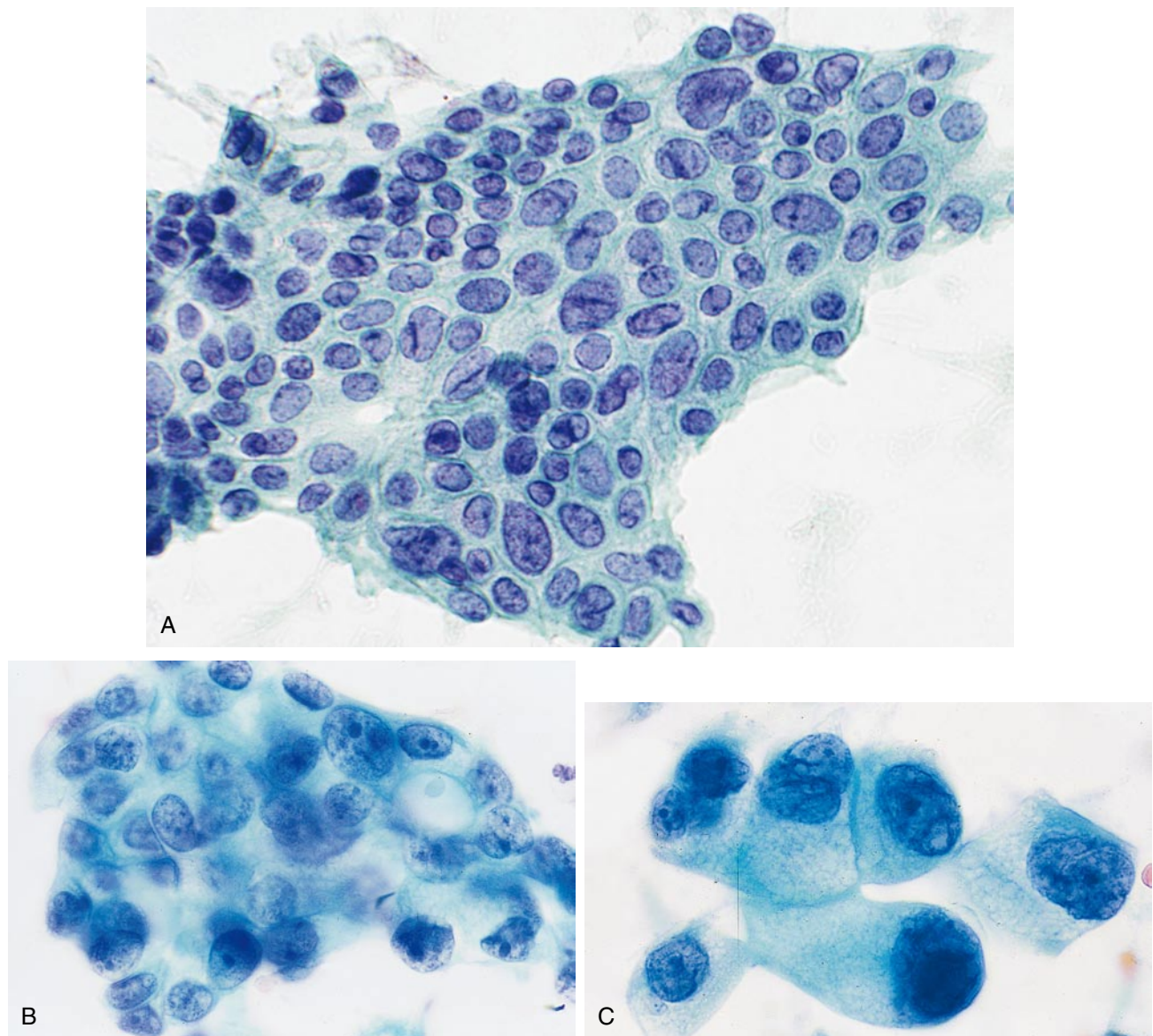


Figure 13.4 Pancreatic ductal adenocarcinoma. A, Compare the appearance of this disordered, crowded sheet with the evenly spaced cells in Figure 13-2A (Papanicolaou stain). B, Marked anisonucleosis is evident (Papanicolaou stain). C, Irregular chromatin distribution and hyperchromasia are seen in moderately and poorly differentiated ductal adenocarcinomas (Papanicolaou stain).

and poorly differentiated cancers. Although a helpful finding in conjunction with the other features previously described, mitoses are not diagnostic of malignancy by themselves because they can be seen with reactive ductal cells responding to injury.²³ Well-differentiated adenocarcinomas often have abundant mucinous cytoplasm; cytoplasm is less copious in moderately and poorly differentiated cancers. The uncommon poorly differentiated ductal adenocarcinomas show marked pleomorphism, abundant mitotic activity, greater cell dyshesion, and some may contain foci of squamous, sarcomatoid, and anaplastic differentiation.

DIFFERENTIAL DIAGNOSIS OF DUCTAL ADENOCARCINOMA:

- chronic pancreatitis
- radiation
- stent atypia

Several benign conditions cause ductal cell atypia that mimics ductal adenocarcinoma. Chronic pancreatitis and radiation to the pancreas often result in some degree of ductal cell atypia, including nuclear enlargement and prominent nucleoli. In both cases, cytologic preparations are sparsely cellular, and there are few if any isolated atypical cells. Biliary and pancreatic duct stents cause irritation and reactive or reparative ductal cell changes, with enlarged nuclei and nucleoli; mitoses may be present. When evaluating a pancreatic or bile duct brushing from a patient with a current or recent stent, bland, moderate nuclear enlargement (without hyperchromasia or membrane irregularity), prominent nucleoli, and even occasional mitoses must be viewed with some skepticism. Nuclear membrane irregularity, an increased nuclear-to-cytoplasmic ratio, isolated atypical cells, and marked anisonucleosis (= fourfold differences in nuclear size) are more reliable as indicators of malignancy. A definite distinction between reactive atypia and ductal adenocarcinoma is not possible in all cases. For cases with equivocal findings, either atypical cells present or suspicious for malignancy is appropriate.

The immunohistochemical expression patterns for SMAD4 (DPC4), p53, and caudal-related homeobox 2 (CDX-2) are helpful in distinguishing among ductal adenocarcinoma, chronic pancreatitis, and (for EUS-guided FNAs) benign gastrointestinal epithelium, particularly if cell block preparations are available. Loss of SMAD4 expression, positive immunoreactivity for p53, and absent or only weak and focal reactivity for CDX-2 are common in ductal adenocarcinoma. By contrast, the cells of chronic pancreatitis are immunoreactive for SMAD4 (DPC4) and negative for p53 and CDX-2. Normal intestinal epithelium is strongly reactive for CDX-2 and expresses SMAD4 (DPC4), but is negative for p53.⁶³

VARIANTS OF DUCTAL ADENOCARCINOMA

The two most common variants of pancreatic ductal adenocarcinoma are *adenosquamous carcinoma* (Fig. 13-5), accounting for 3% to 4% of all pancreatic malignancies, and *undifferentiated (anaplastic) carcinoma*, accounting for 0.3% to 10%.⁵² The cytologic features of the other variants (*mucinous noncystic [colloid] carcinoma*, *hepatoid carcinoma*, *medullary carcinoma*, *signet ring cell carcinoma*, and *undifferentiated carcinoma with osteoclast-like giant cells*) have been less well characterized.

Adenosquamous carcinoma is characterized by mucin-producing glandular elements and an admixed squamous component. By definition, the squamous component must comprise at least 30% of the tumor.⁵³ A cytologic diagnosis of malignancy is usually straightforward, but it may be difficult to rule out a metastasis from a lung cancer or another primary.

FNA of *undifferentiated (anaplastic) carcinoma* yields highly cellular smears composed of pleomorphic epithelioid and spindle cells and bizarre multinucleated tumor giant cells. The cells are poorly cohesive and arranged in a background of necrosis and inflammatory infiltrate. Phagocytosis of inflammatory cells and red blood cells is a striking feature.⁷⁰ Foci of glandular differentiation are present in virtually all cases but they are not always sampled (or readily apparent) by FNA. The differential diagnosis includes poorly differentiated endocrine carcinoma, melanoma, the pleomorphic sarcomas, and choriocarcinoma. A metastasis from an anaplastic lung or thyroid carcinoma must also be excluded.

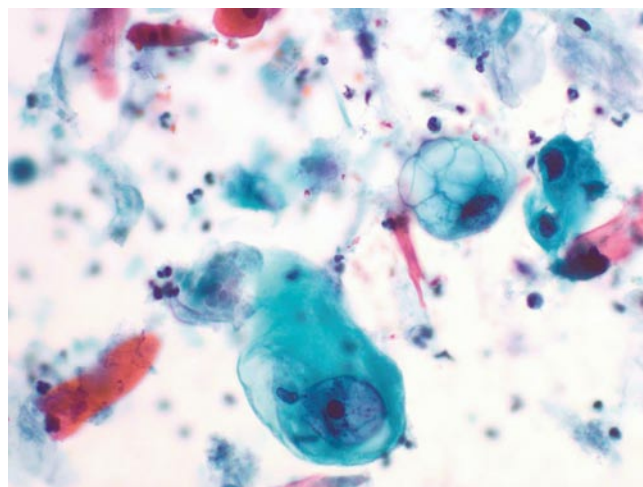


Figure 13.5 Adenosquamous carcinoma of the pancreas. These tumors are defined by a combination of malignant glandular and squamous elements. The malignant squamous component, which sometimes predominates, is characterized by dense, sometimes orangeophilic cytoplasm (Papanicolaou stain).

ACINAR CELL CARCINOMA

ACC is a tumor that phenotypically resembles pancreatic acini and produces pancreatic enzymes. It comprises less than 2% of all pancreatic exocrine tumors and tends to occur in older adults (mean age 62 years), but it is occasionally also seen in children and adolescents. It has a poor prognosis, with an overall 5-year survival that is less than 10%.⁷¹ Tumors range in size but can be quite bulky. They differ from ductal adenocarcinomas in that they are usually circumscribed, and they can be multinodular and multicystic. Histologically, the malignant cells are usually arranged in an acinar or solid pattern and contain scant to abundant granular cytoplasm. Nuclei are round or oval and quite uniform, and typically have a single prominent nucleolus.

CYTOMORPHOLOGY OF ACINAR CELL CARCINOMA:

- highly cellular specimen
- loose cell aggregates
- numerous isolated cells
- round or oval nucleus
- smooth nuclear contour
- prominent nucleolus
- delicate granular cytoplasm

FNA yields loose aggregates, crowded acinar-like arrangements, and isolated polygonal cells with moderately abundant granular cytoplasm (Fig. 13-6A).^{72,73} The nuclei are larger than those of normal acinar cells, round to oval, and eccentrically placed. They have smooth nuclear contours, fine to coarse chromatin, and usually one prominent nucleolus. Toluidine blue and periodic acid-Schiff (PAS) stains highlight the cytoplasmic gran-

ules. Immunohistochemical stains for pancreatic enzymes trypsin, lipase, chymotrypsin, and phospholipase A2 are usually positive in acinar cell carcinoma (Fig. 13-6B).

DIFFERENTIAL DIAGNOSIS OF ACINAR CELL CARCINOMA:

- benign acinar cells
- PEN
- SPN

ACC is a challenging diagnosis to make by FNA. Because of its rarity, it is difficult to develop familiarity with the cytologic features of ACC. In addition, there is morphologic overlap between ACC and other pancreatic neoplasms. First, one must distinguish the cells ACC from *benign acinar cells*. This is relatively straightforward because the cells of ACC are extremely dyshesive and do not arrange themselves neatly into the small well-circumscribed acini of normal acinar cells. Moreover, normal acinar cells, unlike the cells of ACC, have inconspicuous nucleoli. More difficult is the distinction from a *PEN*.⁷² The cells of both tumors are typically dyshesive, and both can have abundant granular cytoplasm and a prominent nucleolus. The loose, acinus-like aggregates of ACC cells can resemble the rosettes of a PEN. Reliance on morphology alone, it is clear, is fraught with pitfalls. Immunohistochemistry can play an essential role in leading to the correct diagnosis. Both ACCs and PENs are strongly reactive for keratins. But ACCs are typically reactive for pancreatic enzymes like trypsin (Fig. 13-6B), chymotrypsin, and lipase, whereas PENs are almost always strongly reactive for the neuroendocrine markers synaptophysin and chromogranin. There can be some overlap in the immunophenotypes; the neuroendocrine markers are focally positive in some ACCs. In most cases,

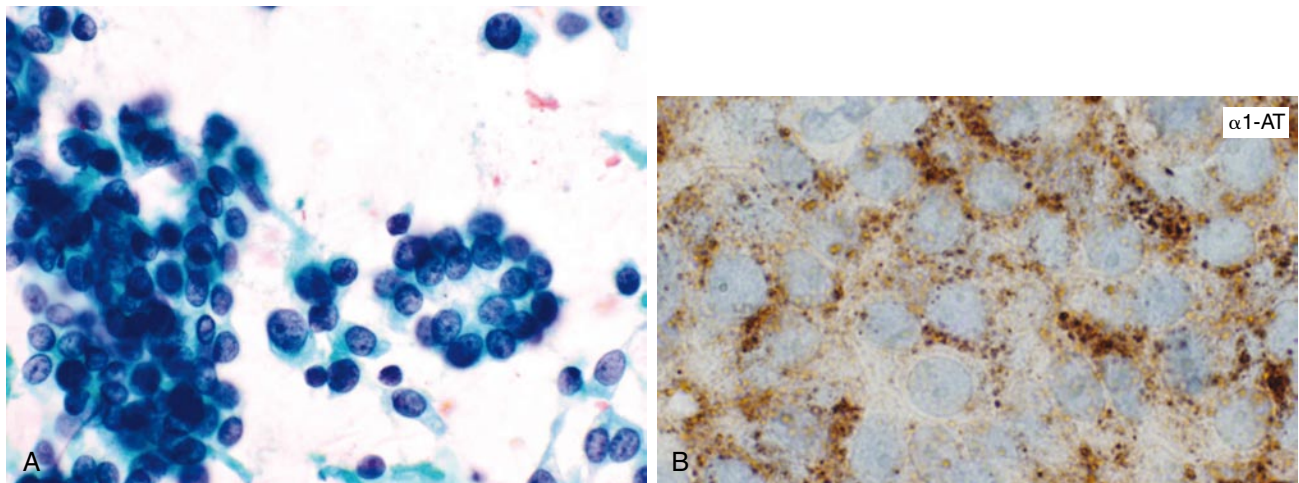


Figure 13.6 Acinar cell carcinoma (ACC). A, Tumor cells are arranged in loose, crowded groups, dispersed single cells, and an occasional rounded structure reminiscent of an acinus. Some nuclei have a prominent nucleolus (Papanicolaou stain). B, The cells are immunoreactive for alpha1-antitrypsin.

however, the immunoprofile can establish the distinction between an ACC and PEN. Another consideration is the SPN. Like PEN, SPN shows considerable morphologic overlap with ACCs, and immunohistochemistry is often essential for the distinction. Most SPNs are negative or only weakly and focally immunoreactive for keratins, but show positive nuclear staining for β -catenin.

SOLID-PSEUDOPAPILLARY NEOPLASM

SPN is an uncommon tumor that accounts for 1% to 2% of PENs. It occurs predominantly in young women (mean age of 35 years) and has no preferential location in the pancreas. Presenting symptoms include abdominal pain and discomfort, but some tumors are discovered incidentally. SPN is considered a tumor of uncertain malignant potential. Most are benign and treated successfully with conservative surgical resection.⁷⁴ Rare tumors do metastasize.⁷⁵ Metastatic potential is associated with perineural invasion, vascular invasion, or invasion into adjacent structures. When these features are identified (usually after surgical resection), the tumor is classified as a *solid-pseudopapillary carcinoma*.

Like ACCs, SPN is usually a well-circumscribed mass. SPNs have varying amounts of solid and cystic components, but cystic spaces, if present, show no septations. Calcifications are sometimes present, and there can be extensive necrosis. Histologically, the tumor is comprised of two intermingled patterns: solid and pseudopapillary arrangements, the latter separated and outlined by red blood cells. Delicate or thickened and hyalinized fibrovascular stalks are a prominent feature. The neoplastic cells show little pleomorphism or anisonucleosis. They have round or oval nuclei, finely textured chromatin, occasional nuclear grooves, and pale or granular cytoplasm.

SPNs are immunoreactive for alpha1-antitrypsin, alpha1-antichymotrypsin, progesterone receptors,⁷⁶⁻⁷⁹ CD56,⁷⁸ CD10,⁷⁸ and KIT (CD117).⁸⁰ Inconsistent results have been reported for epithelial markers (cytokeratins can be positive, but staining is usually weak and focal), synaptophysin, pancreatic enzymes, and islet cell hormones. Recently, an alteration in the adenomatous polyposis coli (APC)/ β -catenin pathway has been identified at the genetic level in SPN, which leads to cytoplasmic/nuclear accumulation of the protein β -catenin in over 95% of tumors.^{81,82} Thus, nuclear immunoreactivity for β -catenin has emerged as a potentially useful adjunct in the diagnosis of SPN.

CYTOMORPHOLOGY OF SOLID-PSEUDOPAPILLARY NEOPLASM:

- highly cellular aspirate
- myxoid or hyalinized vascular stalks lined by neoplastic cells

- delicate granular cytoplasm, indistinct cell borders
- round or oval nuclei
- nuclear grooves
- inconspicuous nucleoli
- foam cells, necrotic debris

FNA yields highly cellular smears composed of a monotonous population of cuboidal cells arranged in loosely cohesive groups, as isolated cells, and, most characteristically, as a single or multiple layer around vascular structures that are often thickened by a myxoid or hyaline material (Fig. 13-7A). The tumor cells have delicate granular cytoplasm with indistinct cell borders. The nuclei are round to oval with finely dispersed chromatin, smooth or grooved nuclear contours, and indistinct nucleoli (Fig. 13-7B). Mitotic figures are usually inconspicuous. The background may contain abundant blood, foam cells, globules of amorphous myxoid material, and necrotic debris.⁸³

The differential diagnosis of SPN includes ACC, and PENs. The characteristic pseudopapillary structures (Fig. 13.7A) with myxoid or hyalinized vascular stalks, along with the typical clinical presentation (usually young women) provide keys to the correct cytologic interpretation. Immunohistochemical studies can be helpful. Most SPNs are negative or only weakly and focally immunoreactive for keratins and synaptophysin, but they usually show nuclear immunoreactivity for β -catenin (Fig. 13.7C).⁸²

PANCREATIC ENDOCRINE NEOPLASMS

Previously called *islet cell tumors*, the term *pancreatic endocrine neoplasm* (or pancreatic endocrine tumor⁸⁴) is favored because some PENs are comprised of endocrine cells not normally found in pancreatic islets (e.g., gastrin-secreting cells). These uncommon tumors represent only 1% to 2% of all pancreatic neoplasms.³ The majority are functional tumors that secrete one of the following hormones: insulin, glucagon, somatostatin, vasoactive intestinal polypeptide (VIP), pancreatic polypeptide, serotonin, adrenocorticotrophic hormone (ACTH), or calcitonin. Resulting from excess hormone secretion, patients with a functional tumor can develop life-threatening symptoms like hypoglycemia, GI ulcers, or diarrhea with dehydration.

PENs occur at any age but are most common in adults. Unlike many pancreatic tumors, they tend to be small, usually about 1 to 5 cm in diameter. Like ACC and SPN, PENs tend to be well-demarcated masses. They can be partially cystic but are completely cystic in only 4% of cases.⁸⁵

Histologically, PENs are divided into well-differentiated and poorly differentiated tumors. Most are well-differentiated, and in common practice, the term *PEN*, when

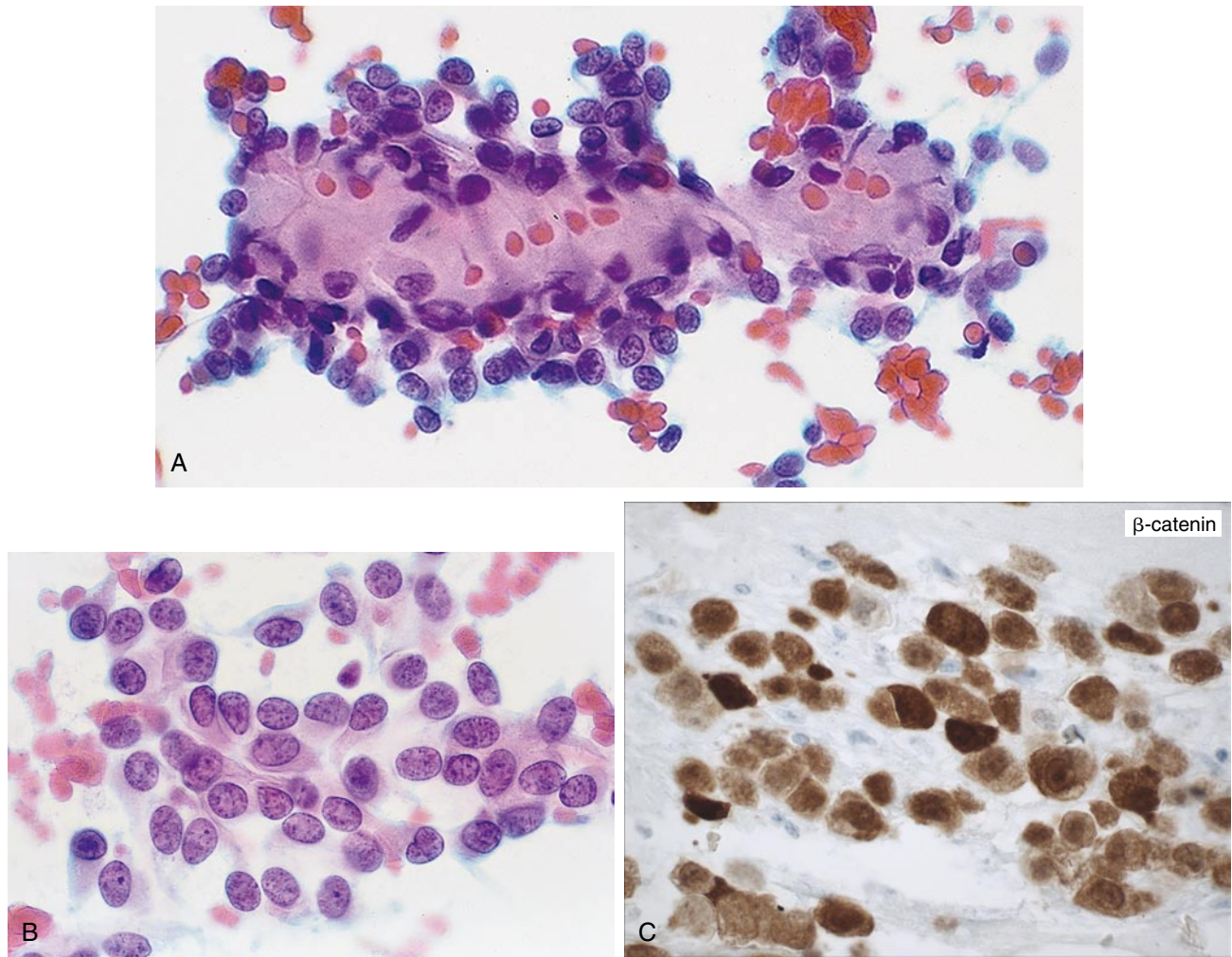


Figure 13.7 Solid-pseudopapillary neoplasm (SPN). A, Tumor cells surround a thick vascular stalk with myxoid change (Papanicolaou stain). B, Tumor cells have granular cytoplasm and round to oval nuclei with finely granular chromatin and an indistinct nucleolus (Papanicolaou stain). C, Tumor cells show nuclear immunoreactivity for β -catenin.

unqualified, is generally assumed to mean a well-differentiated PEN. The cells arrange themselves in a variety of growth patterns (solid, trabecular, and gland-forming). They are usually uniform, with a round or oval nucleus, a distinct nucleolus, and granular or clear cytoplasm. Amyloid deposits are seen with some PENs (especially insulinomas), and somatostatinomas, particularly those that arise in the duodenum, often show gland formation with psammoma bodies. A malignant well-differentiated PEN (well-differentiated endocrine carcinoma) is most reliably identified when there is demonstrable evidence of spread to other organs or infiltration of adjacent structures.^{3,86}

Poorly differentiated endocrine neoplasms or carcinomas are subdivided into *small cell carcinoma* and *large cell endocrine carcinoma*. They are uncommon neoplasms characterized by marked mitotic activity (>10 per 10 high power fields) and immunohistochemical evidence of endocrine differentiation.

CYTOMORPHOLOGY OF WELL-DIFFERENTIATED PANCREATIC ENDOCRINE NEOPLASM:

- highly cellular aspirate
- predominantly isolated cells, bare nuclei
- pseudorosettes
- uniform, round or oval nuclei
- eccentric nuclei ("plasmacytoid" appearance)
- finely stippled ("salt-and-pepper") chromatin
- moderate to abundant cytoplasm

PENs are usually sampled by FNA rather than duct brushings because they rarely invade the pancreatic ducts. FNA of a well-differentiated PEN yields a highly cellular smear with abundant isolated cells and bare nuclei (Fig. 13.8A), loosely cohesive groups, and pseudorosettes (Fig. 13.8B). Multilayered fragments with delicate capillaries are also seen (Fig. 13.8C). The cells

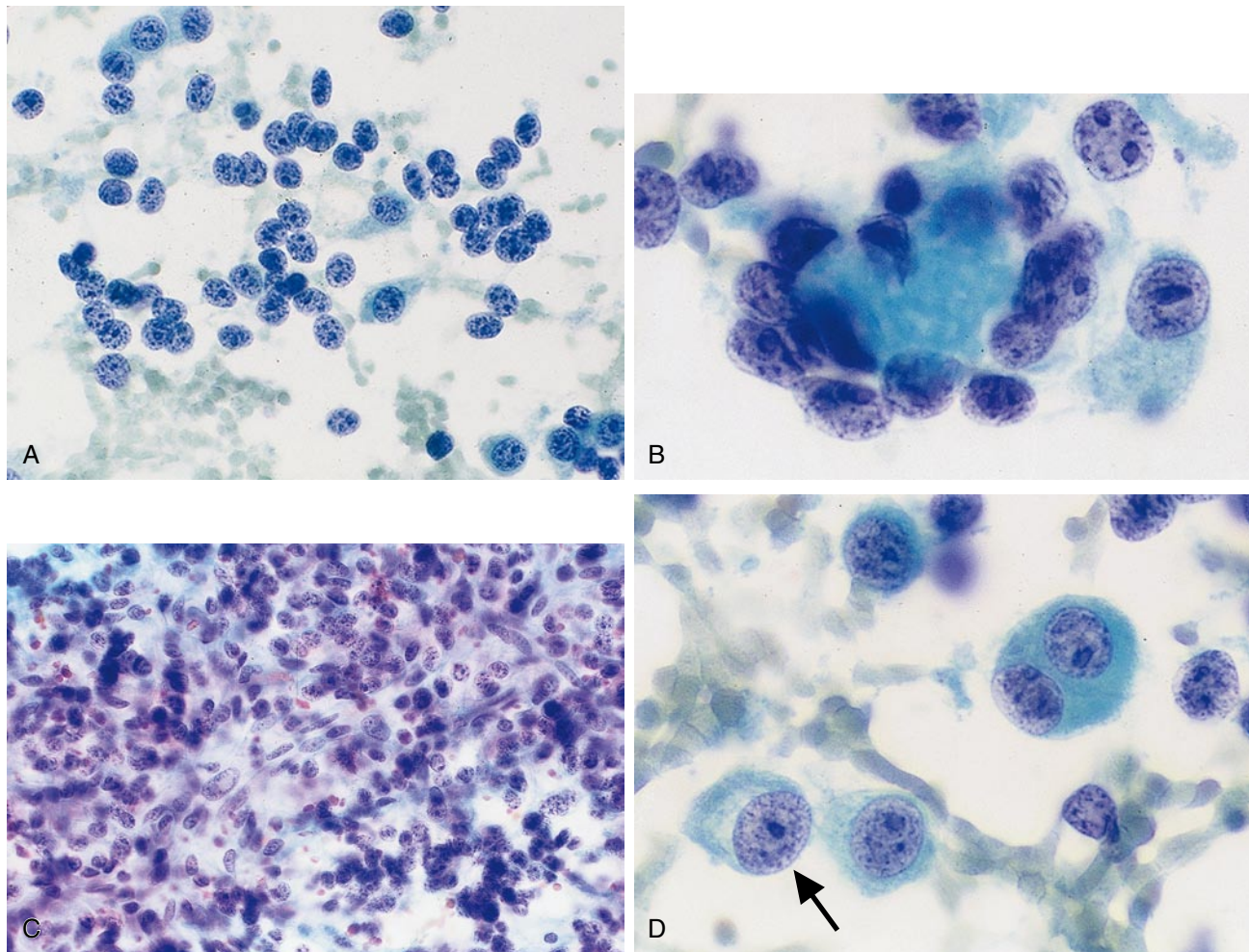


Figure 13.8 Pancreatic endocrine neoplasm (PEN). A, Tumor cells are dispersed as single cells and bare nuclei (Papanicolaou stain). B, Some cells are arranged in a pseudorosette (Papanicolaou stain). C, Large tissue fragments demonstrate delicate traversing vessels (Papanicolaou stain). D, Finely stippled ("salt-and-pepper") chromatin, occasional binucleation, and a plasmacytoid appearance (arrow) are characteristic (Papanicolaou stain).

are monotonous, with an intermediate-sized, round to oval nucleus that is often eccentrically positioned within the cell, imparting a plasmacytoid appearance (Fig. 13.8D), and binucleate cells are present. The chromatin is finely stippled and evenly dispersed ("salt and pepper"). Nucleoli can be prominent. The cytoplasm is finely granular or dense, with ill-defined cell borders; red cytoplasmic granules are seen with Romanowsky stains. A rare mitotic figure or a rare large, pleomorphic cell can be present, but these are not indicative of malignant behavior.³ Poorly differentiated endocrine carcinomas are uncommon and have obviously malignant features (abundant marked pleomorphism and mitotic activity).

DIFFERENTIAL DIAGNOSIS OF WELL-DIFFERENTIATED PANCREATIC ENDOCRINE NEOPLASM:

- ACC
- SPN

- metastatic neuroendocrine carcinoma
- non-Hodgkin lymphoma
- melanoma
- plasmacytoma

The differential diagnosis of a well-differentiated PEN includes ACC and SPN. A distinction based on morphologic features is fraught with hazards because there is much overlap in the cytologic appearance of these neoplasms. Immunohistochemistry, therefore, is often invaluable. Most importantly, PENs are positive for chromogranin and synaptophysin. Some PENs also express pancreatic hormones (e.g., insulin, glucagon, somatostatin, VIP, or gastrin), but correlation between immunohistochemical expression and clinical symptoms is imprecise, and tissue documentation of hormone expression is rarely needed.

In addition to ACC and SPN, the differential diagnosis includes primary or metastatic undifferentiated

neuroendocrine (small cell) carcinoma. PENs lack the necrosis, mitotic activity, and nuclear molding typical of small cell neuroendocrine carcinomas. The isolated cell pattern of a PEN recalls the appearance of some non-Hodgkin lymphomas, but PENs lack the lymphoglandular bodies typical of lymphoid proliferations. Immunohistochemical stains for S-100 protein, HMB45, leukocyte common antigen, and κ and λ light chains can help to distinguish PEN from melanoma and plasmacytoma.

If there is no clinical or pathologic evidence of malignant behavior, well-differentiated PENs are best reported under the general category neoplastic cells present.

The two variants of poorly differentiated endocrine carcinoma have markedly different cytologic appearances. *Small cell carcinoma* resembles small cell carcinoma of the lung, with small cells, scant cytoplasm, nuclear molding, mitoses, and necrosis. *Large cell endocrine carcinoma* (LCEC) more closely resembles a well-differentiated PEN, except that the cells are larger, with more pleomorphism and mitotic activity. Some LCECs resemble poorly differentiated ductal adenocarcinoma. Documenting expression of the neuroendocrine markers synaptophysin and chromogranin is important in confirming the diagnosis of LCEC. The differential diagnosis of poorly differentiated endocrine carcinoma also includes metastatic tumors. Because similar tumors are much more common in the lungs, the possibility of secondary involvement of the pancreas by a lung primary must be considered. It is not clear if immunohistochemical staining for thyroid transcription factor-1 (TTF-1) is helpful in this distinction because few small cell carcinomas of the pancreas have been tested for TTF-1.⁸⁷

SEROUS CYSTADENOMA

Serous cystadenoma is a rare neoplasm that accounts for 1% to 2% of pancreatic tumors. It is subclassified into two types based on the number and size of its cysts. *Serous microcystic adenoma* is composed of numerous small cysts, whereas *serous oligocystic adenoma* has fewer but larger cysts. Serous microcystic adenoma is the more common of the two types. It has a predilection for women, with a mean age at presentation of 66 years, and occurs most often in the body and tail of the pancreas. It is usually benign, but rare cases of serous cystadenocarcinoma have been reported.^{88,89} Some patients present with abdominal pain or other less specific symptoms, but many are discovered incidentally. The numerous small cysts impart a spongy appearance to the tumor on imaging studies. Serous oligocystic adenomas are much less common and are encountered in children and adults. Aside from the size of the cysts, the tumors are morphologically quite similar. Histologically, the cysts are filled with serous fluid and lined by uniform cuboidal

cells with clear, glycogen-rich cytoplasm. Atypia and mitoses are generally absent.

CYTOMORPHOLOGY OF SEROUS CYSTADENOMA:

- sparse cellularity
- clean background or granular material
- flat sheets and loose clusters
- cuboidal cells
- clear or granular cytoplasm with indistinct borders
- bare nuclei
- small round nucleus
- fine chromatin
- inconspicuous nucleolus

Cytologic preparations are characterized by low cellularity and a relatively clean background. The cells are arranged in small clusters or flat sheets. The cytoplasm is clear or vacuolated and has indistinct cytoplasmic borders. The presence of glycogen is demonstrated by PAS positivity that is abolished by predigestion with diastase. The nuclei are small and round with evenly distributed chromatin and an inconspicuous nucleolus. Occasional cases show mild nuclear atypia.⁹⁰

The cytologic diagnosis of serous cystadenoma is quite challenging. Because FNAs are often sparsely cellular, a nondiagnostic or nonspecific negative interpretation is common.⁹⁰ The epithelium is bland and is easily confused with benign pancreatic ductal or acinar epithelial cells or mesothelial cells. There may not be enough cells for cell block preparation and special stains.

DIFFERENTIAL DIAGNOSIS OF SEROUS CYSTADENOMA:

- benign pancreatic ductal and acinar cells
- lymphangioma and hemangioma
- MCN
- cystic pancreatic endocrine neoplasm
- multicystic mesothelioma
- metastatic renal cell carcinoma

Benign acinar cells are arranged in tighter groups than the loose clusters of a serous cystadenoma. Normal ductal cells resemble the cells of a serous cystadenoma, but radiologic correlation may help exclude the possibility that only normal ductal cells were aspirated. Lymphangiomas and hemangiomas are generally lined by flat rather than cuboidal cells; immunohistochemistry for vascular markers can help exclude these possibilities. Sometimes the cells of a serous cystadenoma are taller rather than cuboidal and can be mistaken for a mucinous neoplasm.⁹⁰ This can be especially problematic if the FNA was done endoscopically and the sample is contaminated with mucin of gastric or intestinal contents.

A cystic PEN can be excluded if there is sufficient material for immunohistochemical analysis of neuroendocrine differentiation. Imaging features can help in the distinction from a multicystic mesothelioma, and mesothelial markers (calretinin, Wilms tumor 1 [WT1]) can provide further substantiation. Finally, the clear, glycogen-rich cytoplasm makes serous cystadenomas resemble low-grade renal cell carcinomas. Clinical and imaging correlation can help exclude this possibility.

MUCINOUS CYSTIC NEOPLASM AND INTRADUCTAL PAPILLARY MUCINOUS NEOPLASM

There are two distinct, so-called mucinous neoplasms of the pancreas that share common morphologic features, the *MCN* and the *IPMN*. The distinction between these entities is not possible by cytomorphology alone, but rather it requires correlation with clinical and imaging findings. The distinction is important, however, for clinical management and prognosis. The differential diagnosis includes *ductal adenocarcinoma with mucinous differentiation*. Because MCN and IPMN are morphologically similar, they are discussed together in this section.

MCNs account for 5% of pancreatic neoplasms.⁵² They are found almost exclusively in women (mean age 49 years), and most are located in the body or tail of the pancreas. Imaging studies reveal a sharply defined mass with one or more loculations separated by septae; calcification is sometimes present. An MCN is lined by mucinous epithelium that almost always forms a closed cyst that does not communicate with the ductal system. A defining feature is an ovarian-type stroma that forms a layer of variable thickness beneath the mucinous epithelium and expresses estrogen and progesterone receptors.¹⁵ Histologically, an MCN can fall anywhere along a morphologic spectrum that includes benign, borderline, or frankly malignant (in situ or invasive) epithelium.

IPMN accounts for 3% to 5% of pancreatic tumors.⁵² It is distinguished from ductal adenocarcinoma and MCN because it is an open-ended neoplasm that grows intraluminally along the pancreatic ducts. Like MCN, any given IPMN can be composed of benign, dysplastic, or overtly malignant (in situ or invasive) epithelium. IPMN occurs more commonly in men than women, and 70% are located in the head.⁵² The radiologic features include marked dilatation of the main pancreatic duct and its branches, with no evidence of a pancreatic duct stricture.⁹¹ It can grow diffusely along the entire length of the duct and its branches, or form focal complex papillary proliferations.

The World Health Organization (WHO) classifies MCNs and IPMNs into three types, based on the presence and degree of epithelial dysplasia: benign, borderline, and malignant (noninvasive or invasive). Because of

their malignant potential, surgical resection is often the treatment of choice. Because of the increased sensitivity of imaging techniques, however, more mucinous neoplasms are identified at an early, preinvasive stage, and the decision to resect is nowadays based on a combination of imaging characteristics, the presence or absence of symptoms, the calculated risk of malignancy, and the risk of surgery to the patient.^{92,93} The most important prognostic factor is the presence or absence of invasion. The prognosis for patients with a noninvasive neoplasm is excellent after complete resection regardless of the degree of cytologic atypia.⁵² The prognosis for patients with an invasive MCN or IPMN depends on the depth of invasion, but survival is better than that for patients with an invasive ductal adenocarcinoma. Patients with unresectable disease resulting from local invasion or metastasis have a prognosis similar to that for ductal adenocarcinoma.

Although MCN and IPMN are distinct entities clinically and histologically, they are cytologically indistinguishable. The preoperative distinction between the two neoplasms depends on clinical presentation, location, and imaging characteristics. The role of cytology is to distinguish mucinous from nonmucinous cysts, and to evaluate for the presence or absence of epithelial dysplasia or malignancy.

CYTOLOGY OF MUCINOUS CYSTIC NEOPLASM AND INTRADUCTAL PAPILLARY MUCINOUS NEOPLASM:

- hypocellular specimen
- thick mucin
- columnar mucinous cells (sheets, papillae, or isolated cells)
- nuclear and architectural atypia (if dysplasia or malignancy present)

An FNA of a benign MCN or IPMN (or benign foci within these tumors) is often sparsely cellular, with flat sheets of benign-appearing mucinous epithelium (Fig. 13.9), often in a background of copious thick mucin. (Thinner mucin from stomach or duodenal contents often contaminates EUS-guided specimens.) Distinct cytoplasmic borders within these sheets create a honeycomb-like appearance. The columnar mucinous cells have basally located uniform nuclei and abundant apical cytoplasm. Goblet cells are present in some cases.^{94,95} Because most of these neoplasms are sampled endoscopically, a common dilemma is identifying normal, contaminating gastric or duodenal epithelium (depending on the trajectory) and not misinterpreting these cells as neoplastic. A number of features have been proposed to distinguish neoplastic cyst epithelium from normal gastric or duodenal epithelium. These include thick, colloid-type mucin (characteristic of mucinous

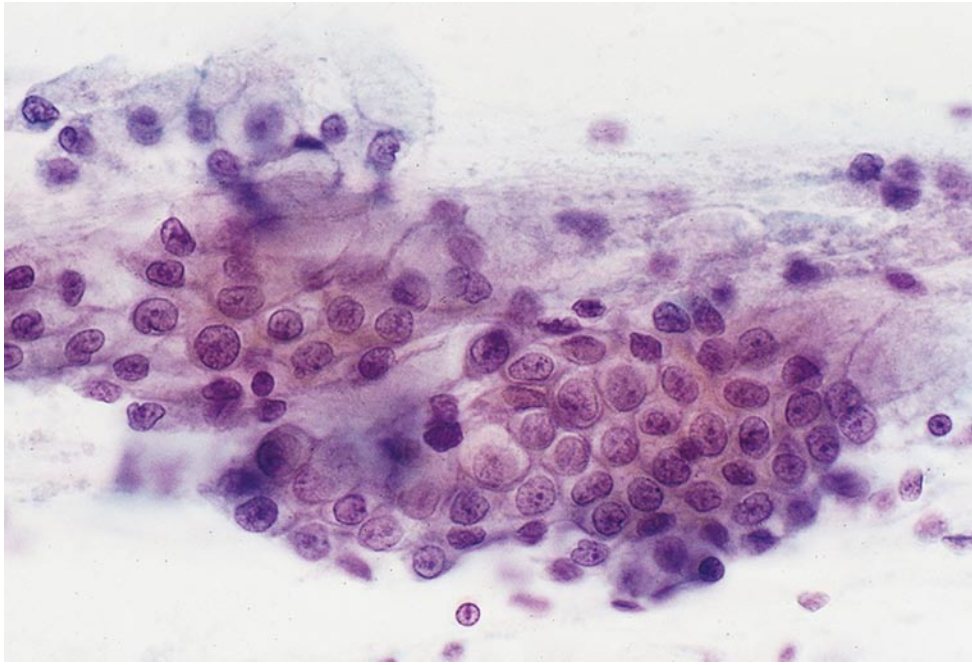


Figure 13.9 Mucinous cystic neoplasm (MCN). Flat sheets of columnar mucinous cells with distinct cytoplasmic borders are characteristic (Papanicolaou stain).

neoplasms),^{96,97} but they have not yet been rigorously tested in a blinded study. The truth is that the distinction between benign neoplastic mucinous epithelium and gastric or duodenal contamination remains treacherous. False-positive interpretations (pseudocysts misinterpreted as mucinous neoplasms as a result of gastric or duodenal contamination) are well-documented.⁹⁴ If one is not convinced that the mucinous epithelium is anything other than normal gastric or duodenal epithelium, then a conservative approach would dictate a nondiagnostic interpretation. If one is convinced that the benign-appearing mucinous epithelium is neoplastic, a negative interpretation should be avoided because these tumors are often heterogeneous, and dysplastic or malignant foci may not have been sampled. Under such conditions, a “neoplastic cells present” categorization is appropriate, followed by “consistent with MCN” or “consistent with IPMN,” depending on the clinical or imaging context. The comment “No evidence of dysplasia or malignancy in this sample” may be helpful, so long as the provider is fully aware that the neoplasm may have been undersampled.

If an MCN or IPMN has foci of dysplasia or malignancy, and if these foci are sampled, a cytologic interpretation is less problematic.⁹⁸ If mucinous epithelium shows atypia in the form of nuclear enlargement, pseudostratification, crowding or overlapping, and micropapillary arrangements, a neoplasm can be confirmed, and mere gastric or duodenal contamination is excluded. Cytologic features mirror histologic grade: necrosis, mitoses, and signet ringlike cells coupled with marked nuclear atypia are indicative of malignancy⁹⁸ and warrant a diagnosis of *mucinous cystadenocarcinoma* or *papillary mucinous carcinoma*.

Although cytology is useful for the preoperative diagnosis of MCN and IPMN, its sensitivity is less than 50%.⁵⁷ Combining cytologic data with that obtained from endoscopic sonography and cyst fluid analysis can aid in clinical decision making.^{92,93} Molecular diagnostics, specifically the identification of the K-ras point mutation and microsatellite marker analysis for loss of heterozygosity, shows promise as an adjunct in the evaluation of mucinous cysts,^{48,50} but additional validation is necessary before it gains widespread application.

SECONDARY PANCREATIC NEOPLASMS

FNA of the pancreas is particularly useful in documenting a metastatic malignancy involving the pancreas. Accurate diagnosis by FNA prompts a thorough clinical work-up to document the primary site and institute appropriate therapy. This allows the patient to avoid the morbidity of unnecessary surgical procedures.

The most common primary neoplasms to secondarily involve the pancreas include carcinomas of the lung (small cell and squamous cell carcinoma), breast, and kidney, and lymphoma.^{99,100} Rare cases of metastatic adenocarcinoma from the ovary, colon, gallbladder, stomach, and prostate have also been reported. Other sources of squamous cell carcinoma include cervix, tonsil, and esophagus. Finally, melanoma, hepatocellular carcinoma, Merkel cell carcinoma, plasmacytoma, and a number of sarcomas are represented in several case reports.¹⁰⁰ Immunohistochemical staining for TTF-1, positive in a large percentage of lung adenocarcinomas and negative in pancreatic adenocarcinomas, helps

distinguish the two adenocarcinomas. It is not clear if TTF-1 is helpful in distinguishing a primary small cell carcinoma of the pancreas from metastatic pulmonary small cell carcinoma, because few pancreatic small cell carcinomas have been evaluated for TTF-1 expression.⁸⁷

Because pancreatic ductal adenocarcinoma is by far the most common malignancy of the pancreas, any aspirate that demonstrates cytologic features unusual for this diagnosis should raise the possibility of a metastasis. Primary squamous cell carcinoma and small cell carcinoma of the pancreas are exceedingly rare. Before these diagnoses are made, alternate primary sites should be excluded clinically. Distinctive cytologic features may permit a definitive diagnosis of metastatic adenocarcinoma from sites such as the prostate, colon, or kidney, but most cases require immunohistochemical stains and a thorough clinical history.

REFERENCES

- Ekberg O, Bergenfeldt M, Aspelin P, et al.: Reliability of ultrasound-guided fine-needle biopsy of pancreatic masses. *Acta Radiol* 1988;29(5):535-539.
- Palazzo L, Roseau G, Gayet B, et al.: Endoscopic ultrasonography in the diagnosis and staging of pancreatic adenocarcinoma. Results of a prospective study with comparison to ultrasonography and CT scan. *Endoscopy* 1993;25(2):143-150.
- Centeno BA: Fine needle aspiration biopsy of the pancreas. *Clin Lab Med* 1998;18(3):401-427, v-vi.
- Brugge WR: Advances in the endoscopic management of patients with pancreatic and biliary malignancies. *South Med J* 2006;99(12):1358-1366.
- Erickson RA, Garza AA: Impact of endoscopic ultrasound on the management and outcome of pancreatic carcinoma. *Am J Gastroenterol* 2000;95(9):2248-2254.
- Jhala NC, Jhala D, Eltoum I, et al.: Endoscopic ultrasound-guided fine-needle aspiration biopsy: A powerful tool to obtain samples from small lesions. *Cancer* 2004;102(4):239-246.
- Eloubeidi MA, Chen VK, Eltoum IA, et al.: Endoscopic ultrasound-guided fine needle aspiration biopsy of patients with suspected pancreatic cancer: Diagnostic accuracy and acute and 30-day complications. *Am J Gastroenterol* 2003;98(12):2663-2668.
- Harewood GC, Wiersema MJ: Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic masses. *Am J Gastroenterol* 2002;97(6):1386-1391.
- Bardales RH, Stelow EB, Mallery S, et al.: Review of endoscopic ultrasound-guided fine-needle aspiration cytology. *Diagn Cytopathol* 2006;34(2):140-175.
- Brugge WR: Pancreatic cancer staging. Endoscopic ultrasonography criteria for vascular invasion. *Gastrointest Endosc Clin N Am* 1995;5(4):741-753.
- Volmar KE, Vollmer RT, Jowell PS, et al.: Pancreatic FNA in 1000 cases: A comparison of imaging modalities. *Gastrointest Endosc* 2005;61(7):854-861.
- Chang KJ, Nguyen P, Erickson RA, et al.: The clinical utility of endoscopic ultrasound-guided fine-needle aspiration in the diagnosis and staging of pancreatic carcinoma. *Gastrointest Endosc* 1997;45(5):387-393.
- Volmar KE, Vollmer RT, Routbort MJ, Creager AJ: Pancreatic and bile duct brushing cytology in 1000 cases: Review of findings and comparison of preparation methods. *Cancer* 2006;108(4):231-238.
- Ryan ME: Cytologic brushings of ductal lesions during ERCP. *Gastrointest Endosc* 1991;37(2):139-142.
- Dodd LG, Farrell TA, Layfield LJ: Mucinous cystic tumor of the pancreas: An analysis of FNA characteristics with an emphasis on the spectrum of malignancy associated features. *Diagn Cytopathol* 1995;12(2):113-119.
- McGuire DE, Venu RP, Brown RD, et al.: Brush cytology for pancreatic carcinoma: an analysis of factors influencing results. *Gastrointest Endosc* 1996;44(3):300-304.
- Logrono R, Kurtycz DF, Molina CP, et al.: Analysis of false-negative diagnoses on endoscopic brush cytology of biliary and pancreatic duct strictures: The experience at 2 university hospitals. *Arch Pathol Lab Med* 2000;124(3):387-392.
- Bardales RH, Stanley MW, Simpson DD, et al.: Diagnostic value of brush cytology in the diagnosis of duodenal, biliary, and ampullary neoplasms. *Am J Clin Pathol* 1998;109:540-548.
- Sheehan MM, Fraser A, Ravindran R, McAteer D: Bile duct brushings cytology—Improving sensitivity of diagnosis using ThinPrep technique: a review of 113 cases. *Cytopathology* 2007;18(4):225-233.
- Brandt KR, Charboneau JW, Stephens DH, et al.: CT- and US-guided biopsy of the pancreas. *Radiology* 1993;187(1):99-104.
- David O, Green L, Reddy V, et al.: Pancreatic masses: A multi-institutional study of 364 fine-needle aspiration biopsies with histopathologic correlation. *Diagn Cytopathol* 1998;19(6):423-427.
- Di Stasi M, Lencioni R, Solmi L, et al.: Ultrasound-guided fine needle biopsy of pancreatic masses: results of a multicenter study. *Am J Gastroenterol* 1998;93(8):1329-1333.
- Robbins D, Katz R, Evans D, et al.: Fine needle aspiration of the pancreas. In quest of accuracy. *Acta Cytol* 1995;39:1-10.
- Gress FG, Hawes RH, Savides TJ, et al.: Endoscopic ultrasound-guided fine-needle aspiration biopsy using linear array and radial scanning endosonography. *Gastrointest Endosc* 1997;45(3):243-250.
- Faigel DO, Ginsberg GG, Bentz JS, et al.: Endoscopic ultrasound-guided real-time fine-needle aspiration biopsy of the pancreas in cancer patients with pancreatic lesions. *J Clin Oncol* 1997;15(4):1439-1443.
- Bentz JS, Kochman ML, Faigel DO, et al.: Endoscopic ultrasound-guided real-time fine-needle aspiration: Clinicopathologic features of 60 patients. *Diagn Cytopathol* 1998;18(2):98-109.
- Voss M, Hammel P, Molas G, et al.: Value of endoscopic ultrasound guided fine needle aspiration biopsy in the diagnosis of solid pancreatic masses. *Gut* 2000;46(2):244-249.
- Eloubeidi MA, Jhala D, Chhieng DC, et al.: Yield of endoscopic ultrasound-guided fine-needle aspiration biopsy in patients with suspected pancreatic carcinoma. *Cancer* 2003;99(5):285-292.
- Varadarajulu S, Tamhane A, Eloubeidi MA: Yield of EUS-guided FNA of pancreatic masses in the presence or the absence of chronic pancreatitis. *Gastrointest Endosc* 2005;62(5):728-736; quiz 751, 753.
- Stelow EB, Bardales RH, Lai R, et al.: The cytological spectrum of chronic pancreatitis. *Diagn Cytopathol* 2005;32(2):65-69.
- Takahashi K, Yamao K, Okubo K, et al.: Differential diagnosis of pancreatic cancer and focal pancreatitis by using EUS-guided FNA. *Gastrointest Endosc* 2005;61(1):76-79.
- Bentz JS: Endoscopic—Ultrasound guided real-time fine needle biopsy. ASC Cytotele conference. Salt Lake City, Utah, ASC, 2001.
- Chang KJ: Endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA) in the USA. *Endoscopy* 1998;30 Suppl 1: A159-A160.

34. Paksoy N, Lilleng R, Hagmar B, Wetteland J: Diagnostic accuracy of fine needle aspiration cytology in pancreatic lesions. A review of 77 cases. *Acta Cytol* 1993;37(6):889-893.
35. Rodriguez J, Kasberg C, Nipper M, et al.: CT-guided needle biopsy of the pancreas: a retrospective analysis of diagnostic accuracy. *Am J Gastroenterol* 1992;87(11):1610-1613.
36. Saleh HA, Khatib G: Positive economic and diagnostic accuracy impacts of on-site evaluation of fine needle aspiration biopsies by pathologists. *Acta Cytol* 1996;40(6):1227-1230.
37. Suits J, Frazee R, Erickson RA: Endoscopic ultrasound and fine needle aspiration for the evaluation of pancreatic masses. *Arch Surg* 1999;134(6):639-642; discussion 642-643.
38. Farrell JJ: Diagnosing pancreatic malignancy in the setting of chronic pancreatitis: Is there room for improvement? *Gastrointest Endosc* 2005;62(5):737-741.
39. Klapman JB, Logrono R, Dye CE, Waxman I: Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. *Am J Gastroenterol* 2003;98(6):1289-1294.
40. Ryan JM, Hahn PF, Mueller PR: Image-guided pancreatic biopsy: Development, indications and complications. *Fine Needle Aspiration Biopsy of the Pancreas*. Boston, Butterworth-Heinemann, 1999.
41. Centeno B, Pitman MB (eds): *Fine Needle Aspiration Biopsy of the Pancreas*. Boston, Butterworth Heinmann, 1999.
42. Levin DP, Bret PM: Percutaneous fine-needle aspiration biopsy of the pancreas resulting in death. *Gastrointest Radiol* 1991;16(1):67-69.
43. Smith EH: Complications of percutaneous abdominal fine-needle biopsy. Review. *Radiology* 1991;178(1):253-258.
44. Livraghi T, Damascelli B, Lombardi C, Spagnoli I: Risk in fine-needle abdominal biopsy. *J Clin Ultrasound* 1983;11(2):77-81.
45. Rashleigh-Belcher HJ, Russell RC, Lees WR: Cutaneous seeding of pancreatic carcinoma by fine-needle aspiration biopsy. *Br J Radiol* 1986;59(698):182-183.
46. Bergenfeldt M, Genell S, Lindholm K, et al.: Needle-tract seeding after percutaneous fine-needle biopsy of pancreatic carcinoma. Case report. *Acta Chir Scand* 1988;154(1):77-79.
47. Dodd LG, Mooney EE, Layfield LJ, Nelson RC: Fine-needle aspiration of the liver and pancreas: A cytology primer for radiologists. *Radiology* 1997;203(1):1-9.
48. Schoedel KE, Finkelstein SD, Ohori NP: K-Ras and microsatellite marker analysis of fine-needle aspirates from intraductal papillary mucinous neoplasms of the pancreas. *Diagn Cytopathol* 2006;34(9):605-608.
49. Khalid A, McGrath KM, Zahid M, et al.: The role of pancreatic cyst fluid molecular analysis in predicting cyst pathology. *Clin Gastroenterol Hepatol* 2005;3(10):967-973.
50. Khalid A, Pal R, Sasatomi E, et al.: Use of microsatellite marker loss of heterozygosity in accurate diagnosis of pancreaticobiliary malignancy from brush cytology samples. *Gut* 2004;53(12):1860-1865.
51. Lewandowski K, Lee J, Southern J, et al.: Cyst fluid analysis in the differential diagnosis of pancreatic cysts: A new approach to the preoperative assessment of pancreatic cystic lesions. *AJR Am J Roentgenol* 1995;164(4):815-819.
52. Hruban RH, Pitman MB, Klimstra DS (eds): *Tumors of the Pancreas*. Washington, DC, American Registry of Pathology, 2007.
53. Hamilton SR, Aaltonen LA (eds): *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Digestive System*. Lyon, France, IARC Press, 2000.
54. Lewandowski KB, Southern JF, Pins MR, et al.: Cyst fluid analysis in the differential diagnosis of pancreatic cysts. A comparison of pseudocysts, serous cystadenomas, mucinous cystic neoplasms, and mucinous cystadenocarcinoma. *Ann Surg* 1993;217(1):41-47.
55. Pinto MM, Meriano FV: Diagnosis of cystic pancreatic lesions by cytologic examination and carcinoembryonic antigen and amylase assays of cyst contents. *Acta Cytol* 1991;35(4):456-463.
56. Hammel P, Levy P, Voitot H, et al.: Preoperative cyst fluid analysis is useful for the differential diagnosis of cystic lesions of the pancreas. *Gastroenterology* 1995;108(4):1230-1235.
57. Brugge WR: Evaluation of pancreatic cystic lesions with EUS. *Gastrointest Endosc* 2004;59(6):698-707.
58. van der Waaij LA, van Dullemen HM, Porte RJ: Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: A pooled analysis. *Gastrointest Endosc* 2005;62(3):383-389.
59. Brugge WR, Lewandowski K, Lee-Lewandowski E, et al.: Diagnosis of pancreatic cystic neoplasms: A report of the cooperative pancreatic cyst study. *Gastroenterology* 2004;126(5):1330-1336.
60. Zhou GX, Huang JF, Li ZS, et al.: Detection of K-ras point mutation and telomerase activity during endoscopic retrograde cholangiopancreatography in diagnosis of pancreatic cancer. *World J Gastroenterol* 2004;10(9):1337-1340.
61. Ponsioen CY, Vrouenraets SM, van Milligen de Wit AM, et al.: Value of brush cytology for dominant strictures in primary sclerosing cholangitis. *Endoscopy* 1999;31:305-309.
62. Lal A, Okonkwo A, Schindler S, et al.: Role of biliary brush cytology in primary sclerosing cholangitis. *Acta Cytol* 2004;48(1):9-12.
63. Shen J, Cibas ES, Qian X: The immunohistochemical expression pattern of SMAD4, p53, and CDX-2 is helpful in diagnosing pancreatic adenocarcinoma in endoscopic ultrasound-guided fine needle aspirations (EUS-FNA). *Mod Pathol* 2007;20(Supplement 2):83A.
64. Warshaw AL, Compton CC, Lewandowski K, et al.: Cystic tumors of the pancreas. New clinical, radiologic, and pathologic observations in 67 patients. *Ann Surg* 1990;212(4):432-443; discussion 444-445.
65. Policarpo-Nicolas ML, Shami VM, Kahaleh M, et al.: Fine-needle aspiration cytology of pancreatic lymphoepithelial cysts. *Cancer* 2006;108(6):501-506.
66. Cohen MB, Egerter DP, Holly EA, et al.: Pancreatic adenocarcinoma: Regression analysis to identify improved cytologic criteria. *Diagn Cytopathol* 1991;7(4):341-345.
67. Francillon Y, Bagby J, Abreo F, Turbat-Herrera E: Criteria for predicting malignancy in fine needle aspiration biopsies (FNAB) of the pancreas and biliary tree. *Acta Cytol* 1996;40:1084.
68. Mitchell ML, Carney CN: Cytologic criteria for the diagnosis of pancreatic carcinoma. *Am J Clin Pathol* 1985;83(2):171-176.
69. DeMay RM: *The Art and Science of Cytopathology*. Chicago, American Society of Clinical Pathologists, 1996.
70. Silverman JF, Dabbs DJ, Finley JL, Geisinger KR: Fine-needle aspiration biopsy of pleomorphic (giant cell) carcinoma of the pancreas. Cytologic, immunocytochemical, and ultrastructural findings. *Am J Clin Pathol* 1988;89(6):714-720.
71. Samuel LH, Frierson HF Jr: Fine needle aspiration cytology of acinar cell carcinoma of the pancreas: A report of two cases. *Acta Cytol* 1996;40(3):585-591.
72. Stelow EB, Bardales RH, Shami VM, et al.: Cytology of pancreatic acinar cell carcinoma. *Diagn Cytopathol* 2006;34(5):367-372.
73. Labate AM, Klimstra DL, Zakowski MF: Comparative cytologic features of pancreatic acinar cell carcinoma and islet cell tumor. *Diagn Cytopathol* 1997;16(2):112-116.
74. Zinner MJ: Solid and papillary neoplasms of the pancreas. *Surg Clin North Am* 1995;75(5):1017-1024.

75. Cappellari JO, Geisinger KR, Albertson DA, et al.: Malignant papillary cystic tumor of the pancreas. *Cancer* 1990;66(1):193-198.
76. Pettinato G, Manivel JC, Ravetto C, et al.: Papillary cystic tumor of the pancreas. A clinicopathologic study of 20 cases with cytologic, immunohistochemical, ultrastructural, and flow cytometric observations, and a review of the literature. *Am J Clin Pathol* 1992;98(5):478-488.
77. Lompo O, Hofman V, Soler C, et al.: [Solid and pseudopapillary tumor of the pancreas: immunohistochemical and ultrastructural study of 2 pediatric cases]. *Ann Pathol* 2000;20(3):221-224.
78. Notohara K, Hamazaki S, Tsukayama C, et al.: Solid-pseudopapillary tumor of the pancreas: immunohistochemical localization of neuroendocrine markers and CD10. *Am J Surg Pathol* 2000;24(10):1361-1371.
79. Kosmahl M, Seada LS, Janig U, et al.: Solid-pseudopapillary tumor of the pancreas: Its origin revisited. *Virchows Arch* 2000;436(5):473-480.
80. Cao D, Antonescu C, Wong G, et al.: Positive immunohistochemical staining of KIT in solid-pseudopapillary neoplasms of the pancreas is not associated with KIT/PDGFR mutations. *Mod Pathol* 2006;19(9):1157-1163.
81. Abraham SC, Klimstra DS, Wilentz RE, et al.: Solid-pseudopapillary tumors of the pancreas are genetically distinct from pancreatic ductal adenocarcinomas and almost always harbor beta-catenin mutations. *Am J Pathol* 2002;160(4):1361-1369.
82. Tanaka Y, Kato K, Notohara K, et al.: Frequent beta-catenin mutation and cytoplasmic/nuclear accumulation in pancreatic solid-pseudopapillary neoplasm. *Cancer Res* 2001;61(23):8401-8404.
83. Pelosi G, Iannucci A, Zamboni G, et al.: Solid and cystic papillary neoplasm of the pancreas: A clinico-cytopathologic and immunocytochemical study of five new cases diagnosed by fine-needle aspiration cytology and a review of the literature. *Diagn Cytopathol* 1995;13(3):233-246.
84. DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds): *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Endocrine Organs*. Lyon, France, IARC Press, 2004.
85. Iacono C, Serio G, Fugazzola C, et al.: Cystic islet cell tumors of the pancreas. A clinico-pathological report of two nonfunctioning cases and review of the literature. *Int J Pancreatol* 1992;11(3):199-208.
86. Collins BT, Cramer HM: Fine-needle aspiration cytology of islet cell tumors. *Diagn Cytopathol* 1996;15(1):37-45.
87. Ordóñez NG: Value of thyroid transcription factor-1 immunostaining in distinguishing small cell lung carcinomas from other small cell carcinomas. *Am J Surg Pathol* 2000;24(9):1217-1223.
88. George DH, Murphy F, Michalski R, Ulmer BG: Serous cystadenocarcinoma of the pancreas: A new entity? *Am J Surg Pathol* 1989;13(1):61-66.
89. Yoshimi N, Sugie S, Tanaka T, et al.: A rare case of serous cystadenocarcinoma of the pancreas. *Cancer* 1992;69(10):2449-2453.
90. Huang P, Staerckel G, Sneige N, Gong Y: Fine-needle aspiration of pancreatic serous cystadenoma: Cytologic features and diagnostic pitfalls. *Cancer* 2006;108(4):239-249.
91. Lichtenstein DR, Carr-Locke DL: Mucin-secreting tumors of the pancreas. *Gastrointest Endosc Clin N Am* 1995;5(1):237-258.
92. Brugge WR: Cystic pancreatic lesions: Can we diagnose them accurately what to look for? FNA marker molecular analysis resection, surveillance, or endoscopic treatment? *Endoscopy* 2006;38 Suppl 1:S40-S47.
93. Tanaka M, Chari S, Adsay V, et al.: International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. *Pancreatol* 2006;6(1-2):17-32.
94. Emerson RE, Randolph ML, Cramer HM: Endoscopic ultrasound-guided fine-needle aspiration cytology diagnosis of intraductal papillary mucinous neoplasm of the pancreas is highly predictive of pancreatic neoplasia. *Diagn Cytopathol* 2006;34(7):457-462.
95. Sole M, Iglesias C, Fernandez-Esparrach G, et al.: Fine-needle aspiration cytology of intraductal papillary mucinous tumors of the pancreas. *Cancer* 2005;105(5):298-303.
96. Nagle JA, Wilbur DC, Pitman MB: Cytomorphology of gastric and duodenal epithelium and reactivity to B72.3: A baseline for comparison to pancreatic lesions aspirated by EUS-FNAB. *Diagn Cytopathol* 2005;33(6):381-386.
97. Stelow EB, Stanley MW, Bardales RH, et al.: Intraductal papillary-mucinous neoplasm of the pancreas. The findings and limitations of cytologic samples obtained by endoscopic ultrasound-guided fine-needle aspiration. *Am J Clin Pathol* 2003;120(3):398-404.
98. Michaels PJ, Brachtel EF, Bounds BC, et al.: Intraductal papillary mucinous neoplasm of the pancreas: Cytologic features predict histologic grade. *Cancer* 2006;108(3):163-173.
99. Benning TL, Silverman JF, Berns LA, Geisinger KR: Fine needle aspiration of metastatic and hematologic malignancies clinically mimicking pancreatic carcinoma. *Acta Cytol* 1992;36(4):471-476.
100. Carson HJ, Green LK, Castelli MJ, et al.: Utilization of fine-needle aspiration biopsy in the diagnosis of metastatic tumors to the pancreas. *Diagn Cytopathol* 1995;12(1):8-13.

Kidney and Adrenal Gland

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THE ADRENAL GLAND

Specimen Collection, Preparation, and Accuracy

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THE KIDNEY

Fine-needle aspiration (FNA) of the kidney is a useful technique for the diagnosis of selected renal lesions. FNA, as it turns out, is not necessary for most renal masses. In adults, the great majority of renal lesions are either radiologically benign cysts requiring no treatment or radiologically malignant masses for which FNA is redundant. Cross-sectional imaging studies, like ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI), are remarkably accurate in diagnosing most benign cysts and renal cell carcinomas (RCCs). Thus, only a small percentage of adult renal lesions, perhaps less than 10%, are candidates for FNA,¹ but in these cases FNA plays a vital role in patient management.

Until recently, aspiration of a suspected Wilms tumor, the most common renal tumor in children, resulted automatically in clinical upstaging, and thus a renal FNA in the pediatric population was—and still is—rarely done. The staging criteria have changed recently, and FNA no longer leads to clinical upstaging in patients with Wilms tumor. Whether this will eventually result in an increased use of FNA in pediatric patients is unclear because interpretation of these specimens is challenging.^{2,3}

A fine-needle aspiration is indicated when:

- radical nephrectomy is contraindicated
 - probable advanced-stage (unresectable) RCC
 - possible metastasis to the kidney (e.g., history of lung cancer)
 - patient has coincident medical problems
 - patient desires an investigational treatment (lesion ablation in vivo)
- radiologic findings are equivocal
 - atypical cyst
 - small solid mass (“low-fat” angiomyolipoma versus oncocytoma versus RCC)
- a partial nephrectomy is considered
 - small lesion or young patient
 - decreased renal function
- an infection (pyelonephritis or abscess) is suspected

For patients who are not candidates for nephrectomy, FNA provides the easiest method to obtain a diagnosis. A mass might be unresectable for a variety of reasons: (1) based on imaging features, it is probably an advanced-stage RCC; (2) there is a history or suspicion of a primary malignancy elsewhere, and the kidney

mass might be a metastasis; or (3) the patient is a poor operative risk because of a comorbid condition. A benign FNA diagnosis (e.g., oncocytoma or angio-myolipoma [AML]) would avoid unnecessary surgery in such a high-risk patient. In addition, recent investigational protocols involve ablating small RCCs in vivo by cryotherapy, radiofrequency, or ethanol injections; in these patients an FNA is obtained to confirm malignancy before the ablation procedure.⁴

Some patients have a radiographically indeterminate lesion. These include atypical cysts and small, homogeneously enhancing masses. Atypical, radiologically indeterminate cysts, by definition, contain more than a few septations, thickened septae, thick walls, or non-border-forming calcifications. Extensive histologic sampling is needed before one can conclude that an atypical cyst is benign. This significantly limits the use of FNA in this setting; still, FNA is sometimes performed, even though the results need to be viewed with some skepticism.⁵ Small, homogeneously enhancing renal masses are also difficult to classify as benign or malignant by imaging studies alone.⁴ Many turn out to be AMLs with little or no fat content, but some are small RCCs. Because AML can be reliably distinguished from RCC by FNA in most cases, FNA plays an important role in the evaluation of small, homogeneously enhancing renal masses.⁵

Some subtypes of RCC, namely papillary RCC and chromophobe RCC, have a good prognosis and are amenable to treatment by partial nephrectomy. This operation is increasingly common, as the incidence of smaller lesions and lesions in younger patients increases.⁶ Patients with the von Hippel-Lindau (VHL) syndrome, who are at risk for bilateral tumors, and those with decreased renal function are also candidates for nephron-sparing surgery. Although the ultimate decision to treat by partial nephrectomy depends on the size and location of the lesion and other clinical factors, FNA is valuable because it discloses whether or not the tumor is one of the “good prognosis” neoplasms amenable to conservative treatment.

In the patient with focal bacterial pyelonephritis or a renal abscess, needle placement permits both diagnosis and therapeutic drainage. These lesions can appear masslike and mimic a renal tumor. Signs and symptoms of a urinary tract infection (UTI) are usually present, and thus most renal infections can be diagnosed clinically, without the need for an FNA. Some renal infections are clinically subtle, however. Imaging findings sometimes help in the distinction from a neoplasm; pyelonephritis with abscess formation tends to have ill-defined margins and perinephric stranding. If, after clinical and radiographic assessment there is still doubt, FNA can be useful to confirm infection and exclude malignancy.

Specimen Collection and Preparation

Virtually all renal aspirations are performed percutaneously by radiologists using US, CT, or MRI guidance; rarely, a surgeon might obtain an FNA intraoperatively. Complications are uncommon and include bleeding, hematuria, pneumothorax, infection, arteriovenous fistula, and urinoma. Needle track seeding is extremely rare with the use of small (less than 18-gauge) needles; there have been fewer than 10 reported cases of needle track seeding associated with a renal mass FNA, for an estimated incidence of less than 0.01%.⁵

Slides are air-dried and stained with a Romanowsky stain, or alcohol-fixed and stained with the Papanicolaou or hematoxylin and eosin stains. Cell blocks are particularly helpful for identifying architectural features, such as papillae, and provide an ideal platform for immunocytochemical studies (e.g., HMB-45 to confirm the diagnosis of an AML).

Cytogenetics and molecular cytogenetics are useful adjunctive tests, particularly in subtyping RCCs.⁷ A karyotype can be obtained from short-term culture of fresh, needle-rinse fluid. Fluorescence in situ hybridization (FISH) can be performed on unstained cytopins, thinlayers, smears, and cell block sections.

Accuracy

Experienced cytologists find that renal FNA accurately distinguishes benign from malignant lesions in 73% to 94% of cases⁸⁻²²; correct subclassification of RCC is achieved in 74% of cases.²³ Because kidney FNAs are relatively uncommon, it can be difficult for some cytologists to obtain expertise in the evaluation of these specimens. False-positive results have occurred when xanthogranulomatous pyelonephritis, AML, benign hepatocytes, benign tubular cells, glomeruli, and benign adrenal cortical cells have been misinterpreted as a RCC.⁵ Cellularity is important to consider when interpreting a kidney FNA; benign mimics of malignancy can contain atypical cells, but they are usually few in number or the sample itself is hypocellular. Most hypocellular kidney aspirates, therefore, should not be diagnosed as positive even if they contain some atypical cells.

Adequacy

Up to 30% of renal aspirates are non-diagnostic (inadequate); a repeat aspiration is helpful in approximately one half of cases.^{8,9,12,14-17,21} Although there is no consensus on adequacy criteria, it is reasonable to consider a renal FNA specimen adequate if a specific (benign or malignant) diagnosis can be made or if there is sufficient cellularity to suggest a limited differential

diagnosis. A specimen composed exclusively of macrophages (typically from a cystic lesion) is best reported as “non-diagnostic” rather than negative because a cystic RCC cannot be excluded.

Normal Elements

Glomeruli and Tubular Cells

CYTOMORPHOLOGY OF GLOMERULI:

- large, dense, globular structures
- capillary loops

DIFFERENTIAL DIAGNOSIS OF GLOMERULI:

- papillary RCC

CYTOMORPHOLOGY OF PROXIMAL TUBULAR CELLS:

- rare cells, abundant granular cytoplasm

DIFFERENTIAL DIAGNOSIS OF PROXIMAL TUBULAR CELLS:

- oncocytoma
- chromophobe RCC

CYTOMORPHOLOGY OF DISTAL TUBULAR CELLS:

- rare cells, scant granular cytoplasm

DIFFERENTIAL DIAGNOSIS OF DISTAL TUBULAR CELLS:

- low-grade clear cell or papillary RCC

Normal elements are occasionally encountered, particularly when the radiologist is sampling a small lesion and the needle excursions traverse normal kidney. It is vital not to misinterpret normal elements as tumor cells. Glomeruli (Fig. 14.1) are highly cellular globular structures that mimic the papillae of papillary RCC, especially at low magnification. Glomeruli lack atypia, but so do low-grade papillary RCCs. In contrast to papillary RCC, however, the cells in a glomerulus are not evenly distributed, but instead are much denser in the center than at the periphery; most importantly, close inspection of the edges of a glomerulus reveals their distinctive capillary loops.

Proximal tubular cells (Fig. 14.2A) have a round bland nucleus, a small but easily seen nucleolus, and abundant, granular cytoplasm. The cells lack a well-defined

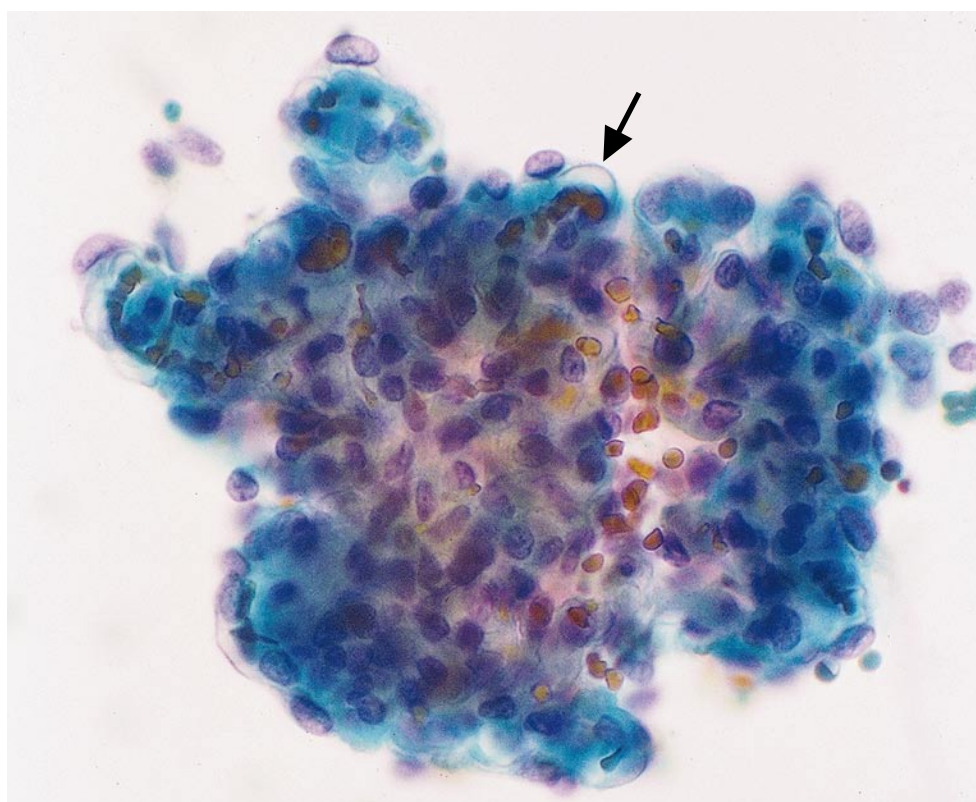


Figure 14.1 Glomerulus. Glomeruli are densely cellular spherical structures with scalloped edges. Note the characteristic capillary loops (arrow; Papanicolaou stain).

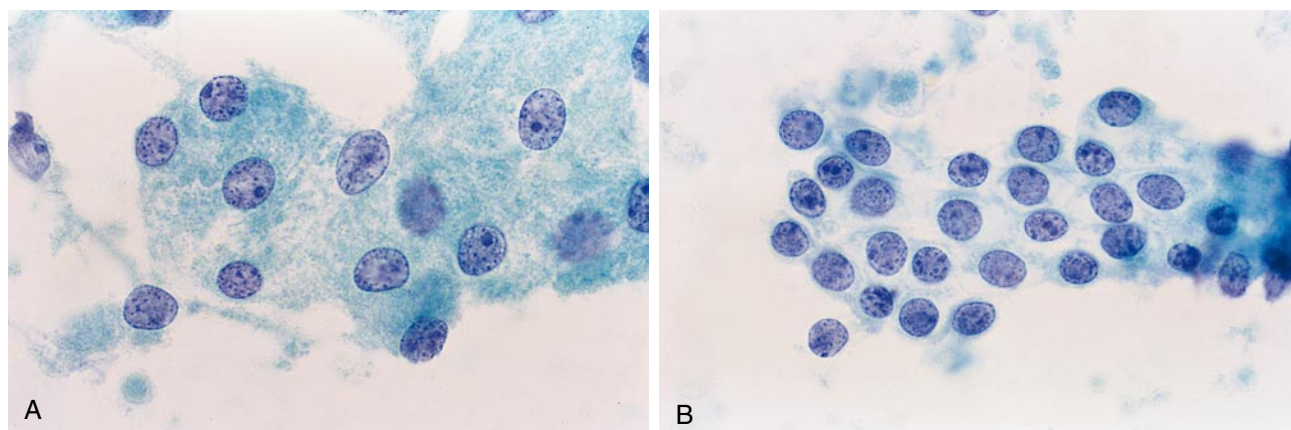


Figure 14.2 Tubular cells. A, Proximal tubular cells have abundant granular cytoplasm and poorly demarcated cell membranes (Papanicolaou stain). B, Distal tubular cells are smaller and have less cytoplasm (Papanicolaou stain).

cell border, and the granules often appear to be spilling out of the cells. They are quite similar to the cells of an oncocytoma and chromophobe RCC. FNAs of both tumors, however, are usually more cellular, and their cells are frequently binucleate, often with some variation in cell and nuclear size and shape. Usually, the cell borders of tumor cells are well defined, while those of proximal tubular cells are torn and irregular.

Distal tubular cells (Fig. 14.2B) are small, isolated or cohesive cells, with less cytoplasm than proximal tubular cells. Their cytoplasm is clear to slightly granular, and they have a small, round nucleus and an inconspicuous nucleolus. The cell borders are well defined, and the cytoplasm is not vacuolated.²⁴ Distal tubular cells are similar to the malignant cells of a low-grade clear cell or papillary RCC. Aspirates of those tumors are usually more cellular, however, and the cells of low-grade papillary RCC form papillae and spherules, something distal tubular cells do not.

Benign Lesions

Oncocytoma

Oncocytoma, a benign tumor of oncocytes (cells with abundant granular cytoplasm), comprises 3% to 5% of all renal tumors;²⁵⁻³¹ rare metastases have been reported.³¹ They have a wide age distribution, with a median size of 6 cm, and most are incidental findings in patients undergoing imaging studies for unrelated reasons. Radiologic findings, unfortunately, cannot be relied on to make a confident diagnosis of oncocytoma. Histologically, tumor cells have abundant granular cytoplasm and uniform round nuclei, with small but distinct nucleoli equivalent to those of a Fuhrman grade 2.³² Occasional cases contain scattered large, sometimes bizarre nuclei, but mitoses are absent or rare. Electron microscopy reveals abundant

mitochondria, which account for the characteristically granular cytoplasm by light microscopy.³³ The arrangement of the neoplastic cells in rounded nests is distinctive and contrasts with the trabeculae (ribbons) of a chromophobe RCC.³⁴ Cytogenetics reveals a mosaic of normal and abnormal karyotypes. The most common abnormalities are losses of chromosomes 1 and Y, but deletions and rearrangements also occur.³⁵ Unfortunately, these findings are non-specific and therefore not useful for confirming the diagnosis of an oncocytoma.

CYTOMORPHOLOGY OF ONCOCYTOMA:

- highly cellular
- rounded nests (cell block)
- cohesive fragments and dyshesive cells (smears)
- abundant uniformly granular cytoplasm
- Fuhrman grade 2 nucleoli

Cytologically, smears from a well-sampled oncocytoma reveal numerous isolated cells with abundant, eosinophilic, granular cytoplasm, well-demarcated cell borders, and round nuclei with small or medium-sized nucleoli³⁶⁻⁴⁰ (Fig. 14.3A). In occasional cases, isolated pleomorphic or bizarre nuclei occur and can be quite alarming,³⁸ but these are thought to represent degenerative changes and are not indicative of malignancy. Necrosis is absent, and mitoses are either absent or infrequent.

DIFFERENTIAL DIAGNOSIS OF ONCOCYTOMA:

- hepatocytes
- clear cell RCC
- papillary RCC
- chromophobe RCC

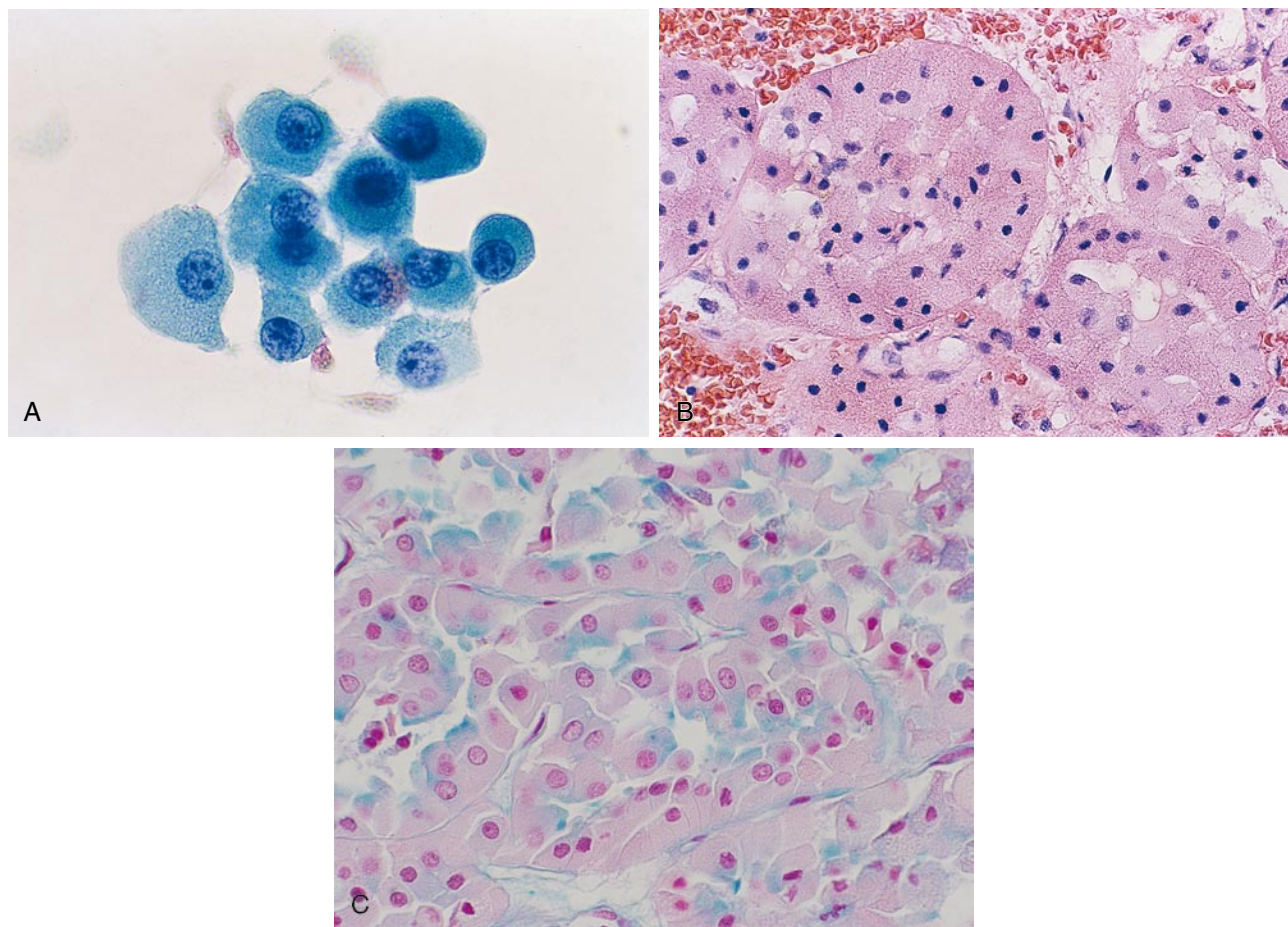


Figure 14.3 Oncocytoma. A, Smears show numerous dyshesive cells with abundant granular cytoplasm and well-demarcated cell membranes. Nucleoli are evident. B, In cell block sections, the round, circumscribed nests characteristic of these tumors are apparent (hematoxylin-eosin [H & E] stain). C, An apical staining pattern with the Hale's colloidal iron (HCI) stain is sometimes encountered with oncocytomas.

When looking at cells with abundant granular cytoplasm in a kidney FNA, one must consider inadvertent sampling of the liver, which happens with FNAs of a right renal mass if the needle traverses the liver on its way to the kidney. Hepatocytes have abundant granular cytoplasm, but they often contain lipofuscin pigment and show more variation in nuclear and cellular size. If hepatocytes are the only finding, the sample is non-diagnostic (insufficient).

One also needs to exclude an RCC. Several different subtypes of RCC can have granular cytoplasm and therefore mimic an oncocytoma. Some clear cell RCCs are comprised mostly of granular (rather than clear) cytoplasm. In comparison to the cells of an oncocytoma, those of a clear cell RCC, including the granular cell variant, are more cohesive, usually with more nuclear atypia and less uniformly granular cytoplasm. The rare eosinophilic variant of papillary RCC has a similar cytologic appearance to an oncocytoma, but papillae and foamy macrophages, typical of papillary RCC, are not features of oncocytomas. The cells of chromophobe RCCs have abundant granular

cytoplasm, but it is usually less uniformly granular (there is often a patchy, perinuclear clearing), and they may have greater nuclear outline irregularity. Nevertheless, in any individual case the distinction between a chromophobe RCC and an oncocytoma can be extremely difficult on smear preparations alone. Tissue fragments in cell block sections, fortunately, are tremendously helpful; the cells of an oncocytoma are arranged in round nests (you can draw a circle around them), whereas the cells of a chromophobe RCC are in endless trabeculae (Fig. 14.3B). The Hale's colloidal iron (HCI) stain can be helpful because it usually shows diffuse cytoplasmic staining in chromophobe RCCs, and most oncocytomas are negative. Unfortunately, some oncocytomas show a positive staining reaction with HCI, but usually only focally and in an apical pattern,⁴¹ and therefore care in interpreting the result is necessary (Fig. 14.3C). Immunohistochemical markers might be helpful in the distinction between oncocytoma, chromophobe RCC, and clear cell RCC, but their reliability in routine practice is not yet known.⁴² To make the situation even more complicated, rare

hybrid renal tumors comprised of oncocytoma and chromophobe RCC do occur, either sporadically or in association with the Birt-Hogg-Dube syndrome⁴³; FNA findings depend on the areas sampled.

If only smears were prepared, the wisest and most honest interpretation in most cases is oncocytic neoplasm (oncocytoma versus chromophobe RCC), with a note that, if clinically indicated, a partial nephrectomy should be considered. This may not satisfy the urologist, who was hoping for an unequivocal interpretation. It is unwise, however, to make a definitive diagnosis of oncocytoma without an assessment of the architecture of the cells, seen with the help of a cell block or core needle biopsy, because a rounded (nested) architecture is essentially the only finding relatively specific for an oncocytoma. Virtually all the other features of oncocytoma can be mimicked by several subtypes of RCCs, not just chromophobe RCC.

Renal Cortical Adenoma

Except for their size, renal cortical adenomas are histologically, immunohistochemically, and cytogenetically indistinguishable from low-grade papillary RCCs.^{44,45} Renal adenomas are by definition small lesions, always less than 0.5cm and usually less than 0.2cm.⁴⁶ They are too small to be aspirated, and therefore the diagnosis renal cell adenoma is not appropriate for an FNA specimen.

Angiomyolipoma

AMLs are benign mesenchymal tumors that arise from the so-called “perivascular epithelioid cell” and comprise between 0.7% and 2.0% of all renal tumors. They occur in two distinct clinical settings.⁴⁷⁻⁴⁹ Approximately one half occur in young adults with tuberous sclerosis (TS), an autosomal dominant disease caused by mutation of one of the two TS-associated genes and manifested by mental retardation, seizures, and skin changes. In patients with TS, the AMLs are usually multiple and bilateral. The other half are usually solitary and occur in young and middle-aged women with no known clinical syndrome. Some patients present with flank pain, but the majority of AMLs are detected incidentally during a radiologic work-up for an unrelated disorder. They range widely in size and can be tiny or up to 20cm in diameter. Large lesions can bleed, and some are resected to prevent this from occurring.

Histologically, an AML is composed of three elements: mature fat, blood vessels, and smooth muscle cells; the latter can have moderate-to-marked atypia. Mature fat makes up the bulk of most AMLs, but these common, fatty AMLs are reliably identified by imaging studies, precluding the need for an FNA. FNA is needed

only for the subset of AMLs that have little adipose tissue (the “fat-free” or “low-fat” AMLs), which cannot be distinguished from an RCC by imaging alone. In virtually all cases, the neoplastic cells, most conspicuously the smooth muscle cells but also the lipid-distended fat cells, are immunoreactive for melanoma-related antigens like HMB-45 and MART-1.⁵⁰⁻⁶⁰ An uncommon subtype of AML, called an epithelioid AML, is composed of large epithelioid (rather than spindle) smooth muscle cells; epithelioid AMLs have metastatic potential, but predicting which will behave in a malignant fashion is problematic.⁵⁰

CYTOMORPHOLOGY OF ANGIOMYOLIPOMA:

- spindled cells (with or without nuclear atypia) with stringy cytoplasm
- fat cells
- thick-walled blood vessels (rare)
- epithelioid cells with marked atypia (“epithelioid AML”)

AMLs can be diagnosed confidently by FNA, but they do have their challenges: Specimens are usually paucicellular; the adipose tissue component is scant or nowhere to be found; and the thick vessels are rarely seen. The smooth muscle cells, sometimes with moderate-to-marked nuclear atypia, usually dominate the picture of an FNA (Fig. 14.4A-C). In an AML, atypical spindle cells and their large nuclei, if present, are usually interspersed among numerous much less atypical cells. The cytoplasm of the smooth muscle cells has a stringy appearance, quite different from the vacuolated or granular cytoplasm of RCC. Scattered large, clear fat vacuoles can be present in the smooth muscle cells of an AML and impart a resemblance to an RCC. The differential diagnosis includes sarcoma⁶¹ and sarcomatoid RCC. Immunoreactivity for HMB-45 and MART-1 is extremely helpful, because RCCs and most sarcomas are negative for these markers (Fig. 14.4D).

In an epithelioid AML, the cells are round and range in size from medium to huge; because of their abundant cytoplasm and enormous nucleoli, they resemble ganglion cells. Necrosis and mitoses can be seen. The differential diagnosis of an epithelioid AML includes clear cell RCC.^{9,60,62} Here, too, immunostains are extremely helpful in distinguishing an epithelioid AML from an RCC. Thus, it is prudent to obtain immunostains on any atypical or paucicellular spindle cell or epithelioid cell renal lesion.

Metanephric Adenoma

Metanephric adenoma (MA) is a rare, essentially benign kidney tumor,⁶³⁻⁶⁶ although the occasional case with metastases has been described.⁶⁷ It can be found

at any age but most commonly occurs in women in the fifth decade. About one half are discovered incidentally; the rest are detected because of hematuria, flank pain, or polycythemia. They range from small to large lesions, and can measure up to 15 cm in diameter. Histologically, MA is composed of tight, uniform tubules lined by bland cells with small round nuclei and inconspicuous nucleoli. Mitoses are absent or infrequent. Psammoma bodies are common. They resemble low-grade papillary RCCs and differentiated Wilms tumors. Unlike papillary RCC, they are negative for epithelial membrane antigen (EMA) and have a normal karyotype. Like Wilms tumor, MAs show nuclear immunoreactivity for Wilms tumor 1 (WT1).

CYTOMORPHOLOGY OF METANEPHRIC ADENOMA:

- tight cell clusters or sheets
- small cells
- scant cytoplasm
- round, uniform nuclei
- small nucleoli

On cytologic preparations, the cells form short tubules, tight balls, and loose sheets; they have scant cytoplasm, round monotonous nuclei, fine, even chromatin, and rare small nucleoli⁶⁸ (Fig. 14.5A-C).

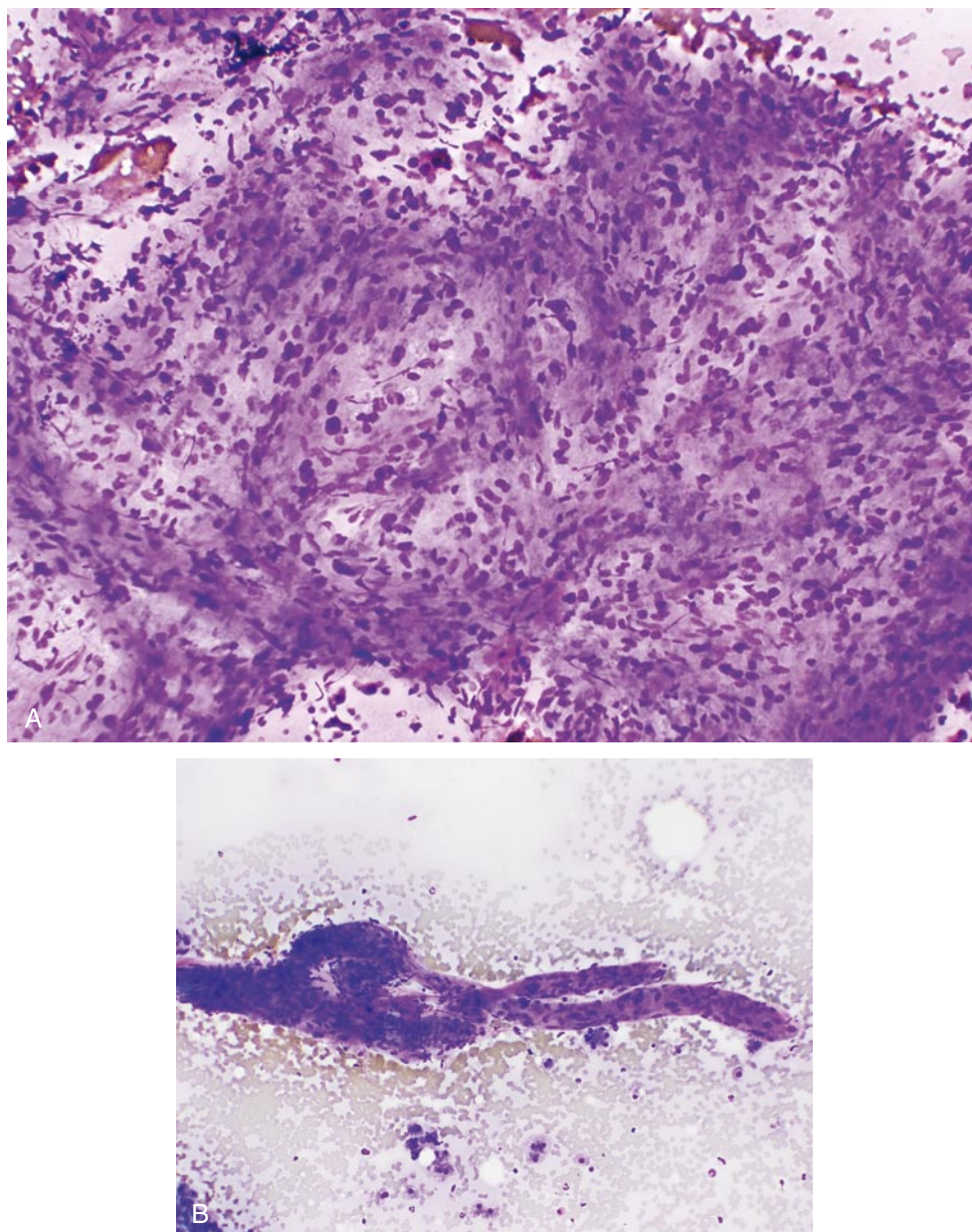


Figure 14.4 Angiomyolipoma. A, Cohesive tissue fragments comprised of spindled-shaped smooth muscle cells are usually the predominant finding (Papanicolaou stain). B, Occasionally, thick-walled blood vessels are seen (Wright-Giemsa stain).

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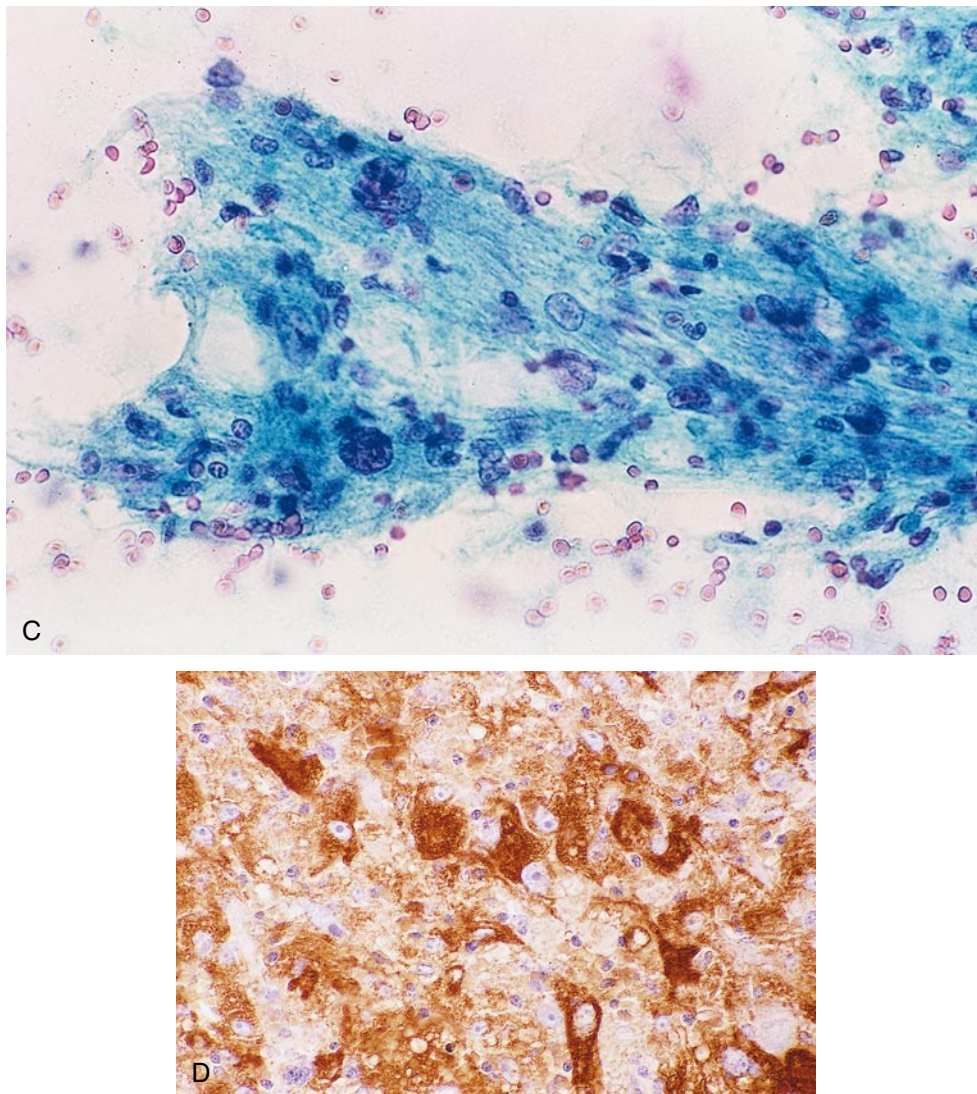


Figure 14.4 (Continued) **Angiomyolipoma.** C, Nuclear atypia is commonly encountered in benign angiomyolipomas (AMLs; Papanicolaou stain). D, The tumor is immunoreactive for HMB-45, a helpful marker in difficult cases.

DIFFERENTIAL DIAGNOSIS OF METANEPHRIC ADENOMA:

- differentiated, epithelial-predominant Wilms tumor
- low-grade papillary RCC
- metastatic tumor

Most Wilms tumors are easily distinguished from MA because Wilms tumors are triphasic neoplasms that contain a blastemal component (small, closely packed, mitotically active cells) as one of their three constituents. An epithelial-predominant Wilms tumor, with little or no blastema, can be virtually impossible to distinguish from a metanephric adenoma,⁶⁹ although some claim—on the basis of histologic material—that the cells of a Wilms tumor are larger, with more hyperchromasia and

mitoses.⁶³ Low-grade papillary RCCs have more cytoplasm than MA and, unlike MA, are positive for EMA and negative for WT 1.^{45,63} Other immunomarkers might be helpful in this distinction as experience with them accrues.⁷⁰ Finally, because of the high nuclear-to-cytoplasmic ratio of MA cells, a metastasis should be considered. Most metastases are EMA-positive, and a metastasis is unlikely in the absence of a known or suspected primary tumor elsewhere.

Cystic Nephroma

Cystic nephroma is a benign cystic neoplasm, also known as a multilocular cyst, that most commonly occurs as a solitary tumor in boys and middle-aged women. It is composed of stroma and small cysts lined by atypical epithelium.⁴⁹ Although cystic, this lesion in fact appears solid radiographically. FNAs are usually

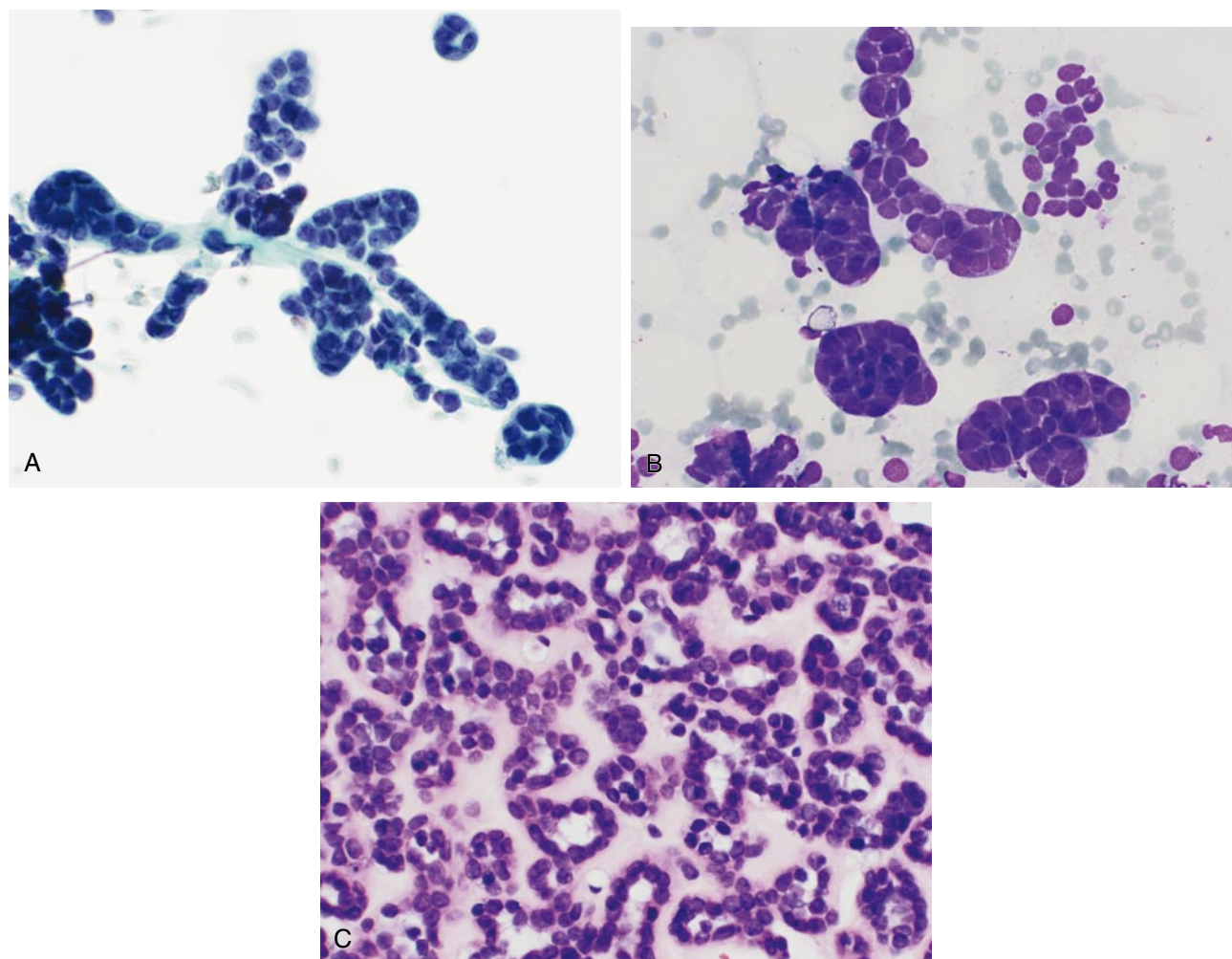


Figure 14.5 Metanephric adenoma. A and B, Smears show short tubules, cords, and tight balls of cells with scant cytoplasm (A, Papanicolaou stain; B, Wright-Giemsa stain). C, Cell block shows numerous small tubules (hematoxylin-eosin [H & E] stain).

misdiagnosed as either RCC, AML, or sarcoma.^{18,71,72} Specimens are generally hypocellular but contain some epithelial cells with clear to vacuolated cytoplasm, nuclear membrane irregularity, and prominent nucleoli.^{73,74} Alternatively, specimens consist of large, pleomorphic spindle cells, admixed with cells with intracytoplasmic vacuoles simulating fat.⁷⁵ As a rule, complete excision is required to make the diagnosis. The only predictable cytologic feature is sparse cellularity. This adds support to the wisdom that hypocellular specimens with atypical cells should be reported as suspicious rather than positive.

Renal Abscess

Focal bacterial pyelonephritis and a renal abscess can appear masslike radiologically.^{1,76} Aspirates contain necrotic material and numerous neutrophils, sometimes with rare atypical cells that are easily confused with those of a clear cell RCC. The atypical cells are few in number, however, and in the context of abundant

acute inflammation should be reported (at most) as suspicious for malignancy.

Xanthogranulomatous Pyelonephritis

Xanthogranulomatous pyelonephritis is an atypical host reaction to a bacterial infection and usually presents as a mass lesion.¹⁷ Histologically and cytologically, the lesion is composed of histiocytes and multinucleated giant cells. The histiocytes can form aggregates and resemble the cells of clear cell RCC, but they lack nuclear atypia and their cytoplasm has a more microvacuolated appearance than that of typical RCCs. Differential immunoreactivity for epithelial membrane antigen and CD68 is helpful in difficult cases.

Renal Infarct

Rarely, renal infarcts have a radiographic appearance suspicious for malignancy.⁷⁷ Specimens are sparsely cellular and composed of necrotic material, which can

contain rare atypical cells resembling those of clear cell RCC. A diagnosis of malignancy should be avoided when the atypical cells are few in number, which is usually the case with renal infarcts.

Renal Cysts

Renal cysts are common. Of all renal lesions 70% to 85% are cysts, and 50% of men over the age of 50 years have at least one cyst.^{78,79} The majority of these cysts are benign, acquired, and solitary; only 1% to 4% of cysts are cystic RCCs,⁸⁰⁻⁸⁶ usually of clear cell or papillary type.⁴⁹ The prognosis of a patient with a cystic RCC is generally excellent,^{87,88} but metastases do occur,⁸⁹ and resection, either by partial or radical nephrectomy, is indicated.

The pretest probability that a renal cyst is malignant depends, in part, on the radiologic appearance. Cysts are classified according to the Bosniak system.⁹⁰⁻⁹⁵ Most lesions are category 1 (benign); category 4 lesions are malignant and are resected directly; and categories 2 and 3 are indeterminate. Between 5% and 57% of indeterminate cysts are malignant. Because the pretest probability of malignancy is as high as 57%, many urologists believe that all indeterminate cysts should be resected.

How helpful is an FNA that lacks atypical cells (contains only macrophages)? Does this result change the pretest probability of an RCC? Much of the data on this subject predates CT and MRI,^{15,76,79,82,83,96-98} but it implies that a negative diagnosis supports the diagnosis of a benign cyst. Closer examination, however, reveals that the vast majority of the lesions in these studies were simple cysts (Bosniak category 1 lesions) that would not be aspirated today. Unfortunately, no study has examined the sensitivity of FNA for Bosniak 2 and 3 lesions. But indirect evidence provides some insights. First, 10% of all RCCs yield a nondiagnostic or falsely negative FNA result.^{9,11,15,18,23,99,100} Second, most RCCs incorrectly interpreted as negative by FNA are cystic.²³ Third, most radiographically suspicious cysts prove to be RCC.^{9,11,18} Finally, in the largest series of cystic RCCs (11 cases), only 2 cases had atypical cells on cytologic examination, and repeat aspirates in both patients were negative.¹⁰¹ From these data, it can be inferred that the sensitivity of FNA for Bosniak category 2 and 3 cysts is low, and certainly no higher than 10% to 20%. Given that the pre-FNA probability is 5% to 57%, a negative FNA result (macrophages only), in this setting, has little effect on the patient's risk of disease and is best reported as non-diagnostic (Fig. 14.6).

Complicating the matter further is the fact that some benign cysts are virtually impossible to distinguish from an RCC by FNA. Patients on renal dialysis for renal failure often acquire cysts, and 9% develop RCC, often a multifocal tumor.¹⁰²⁻¹⁰⁵ Patients with adult polycystic kidney

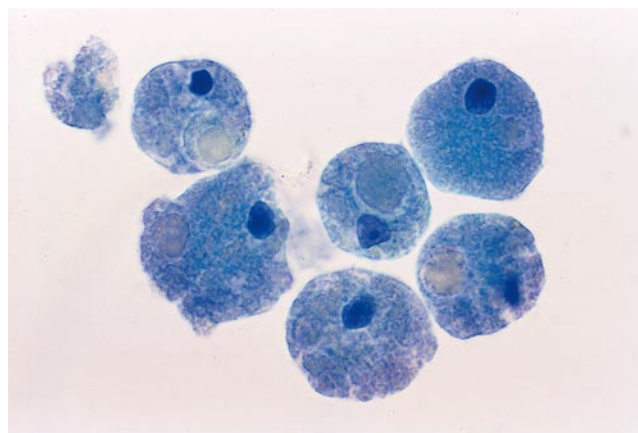


Figure 14.6 Renal cyst. Macrophages are a nonspecific finding (Papanicolaou stain).

disease, an autosomal dominant disease, are also at an increased risk for developing RCC, which, like the tumors in renal dialysis patients, can be multifocal. In both settings, papillary hyperplasia occurs within the cysts,¹⁰⁶ and distinction between this hyperplasia and carcinoma is not possible by FNA. (Histopathologic criteria for this distinction are controversial as well.) The best approach is simply to avoid aspirating cysts in these patients.

When confronted by an FNA of a cystic renal mass, the cytologist can be aided by a review of the imaging findings, especially with a radiologist who has expertise with renal cysts. Some urologists aspirate benign, Bosniak category 1 cysts merely for symptomatic relief. In this setting, where the radiographic probability of RCC is so low, one should take a conservative approach in interpreting any atypical cells. If, on the other hand, the aspirate is performed for an indeterminate (Bosniak category 2 or 3) lesion and even a few atypical cells are seen, a suspicious diagnosis is appropriate (Fig. 14.7). If the result is non-diagnostic (macrophages only), an explanatory note can be helpful, such as "The findings are consistent with a cystic renal lesion. Because parenchymal elements have not been sampled, the possibility of a neoplasm cannot be excluded."

Summary of the challenge of renal cysts:

- Renal cysts are common, and most are benign.
- RCC can be cystic.
- Adequate sampling of a cystic RCC is difficult.
- Some benign cysts are difficult to distinguish from RCC:
 - cysts resulting from renal failure
 - adult polycystic kidney disease
 - cystic nephroma
- The value of a negative diagnosis is limited.

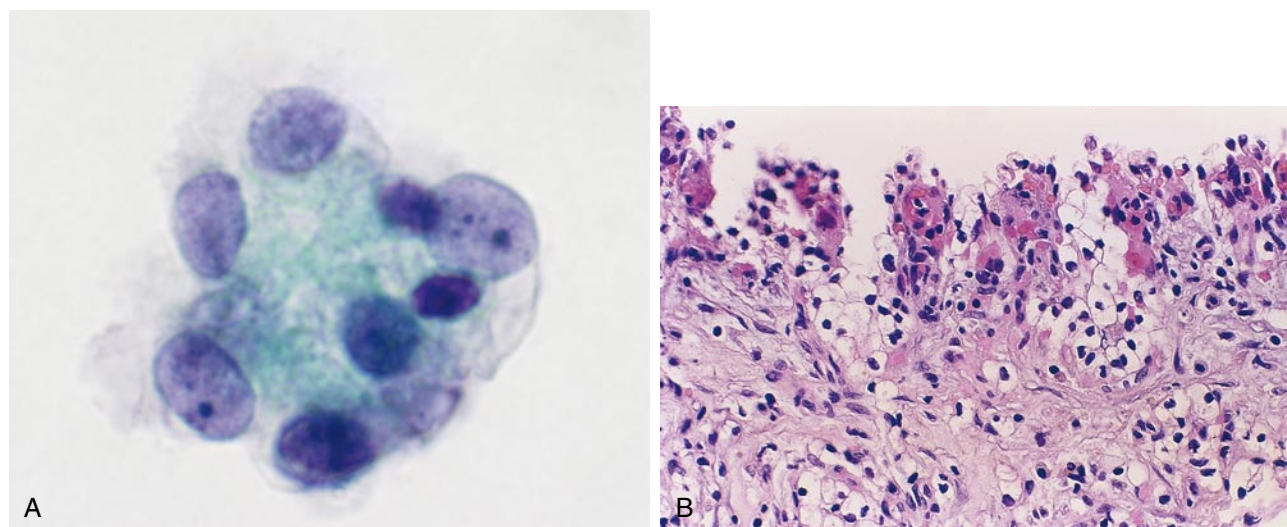


Figure 14.7 Cystic renal cell carcinoma (RCC). A, Fine-needle aspiration (FNA). There were only a few groups of cells with clear or granular cytoplasm on the slide. In the setting of a radiologically atypical cyst, this finding is suspicious for carcinoma. B, Surgical resection. The tumor was almost entirely cystic. Malignant cells were found in the wall in only a few areas (hematoxylin-eosin [H & E] stain).

Atypical cells on a kidney FNA (see summary)

- Benign entities that sometimes have atypical cells:
 - cystic nephroma
 - renal abscess: xanthogranulomatous pyelonephritis
 - renal infarct
 - atypical cysts
 - angiomyolipoma
- Best diagnostic clue:
 - hypocellular specimen
- Best cytologic interpretation:
 - atypical or suspicious (rather than positive)

Renal Cell Carcinoma

Fifty-one thousand malignant tumors of the kidney are diagnosed in the United States every year, with 13,000 deaths.¹⁰⁷ Men are more commonly affected than women; in men, tumors of the kidney are the 10th most frequent cause of cancer-related mortality in the United States, accounting for 3% of all cancer deaths.¹⁰⁷

Of all the malignant tumors of the kidney, RCC, a malignancy of the renal tubules, is the most common. Risk factors include tobacco smoking and obesity. A minority of RCCs occur in the setting of one of several inherited cancer syndromes, the most common being VHL disease. In patients with VHL disease, the mean age of manifestation of RCC is 37 years, as compared with 61 years for sporadic RCC.

Patients sometimes present with the classic triad of hematuria, flank pain, or a palpable mass, but the tumor is often discovered incidentally. The grade of an RCC has prognostic significance second only to tumor stage, and RCCs are graded using the Fuhrman system.³² Because RCC is based on nuclear features, it is easily applied to cytologic preparations, with good cytologic-histologic correlation.^{108,109} With heterogeneous RCCs, the highest grade is assigned.

The most recent histologic classification system,⁴⁶ strongly influenced by genetic evidence, divides RCCs into various subtypes (Table 14.1). Cytologists should be familiar with these subtypes, which differ in their morphologic features and prognosis. When performed on a

TABLE 14.1 SUBTYPES OF RENAL CELL CARCINOMA

Subtype	Percentage of All RCCs	Cytogenetics	Other
Clear cell or conventional	75	del 3p	—
Papillary	15	trisomies 7, 16, 17	Good prognosis, multifocal
Chromophobe	3–5	extensive loss of entire chromosomes (most common: 1, 2, 6, 10, 13, 17, 21)	—
Collecting duct	< 1	not well characterized	—
Sarcomatoid	3	complex karyotypes	Poor prognosis
Translocation-associated	< 1	translocations of X	TFE3+

RCC, renal cell carcinoma; TFE3, transcription factor E3.

portion of the cytologic specimen, cytogenetic or molecular genetic analysis, including conventional karyotyping and FISH, is a helpful adjunct in subclassifying renal neoplasms.

Clear Cell Renal Cell Carcinoma

Clear cell (also called conventional) RCC comprises 75% to 80% of all RCCs and is strongly associated with a variety of deletions on chromosome 3p, the site of the VHL gene.¹¹⁰⁻¹¹³ The average size of a clear cell RCC is 7 cm, but small tumors are being detected with increasing frequency as a result of the increasing use of cross-sectional imaging techniques. Size is not a determinant of malignancy, but the frequency of metastases does correlate with increasing size of the primary tumor. Necrosis, hemorrhage, cystic degeneration, and calcification are common; these features give the clear cell RCC its characteristic heterogeneous appearance on imaging studies. Histologically, clear cell RCC is composed of cells with abundant cytoplasm that is clear, granular, or a mixture of both. The clear and granular appearance is a result of the presence of lipid and glycogen in the cytoplasm. A distinction used to be made between RCCs of predominantly clear or granular type, but this is no longer believed to have clinical relevance, and the granular type is now folded into the clear cell category. The tumor cells have a rich network of delicate, thin-walled blood vessels, which accounts for the contrast enhancement pattern on imaging studies and the frequent bloodiness of FNA samples.

CYTOMORPHOLOGY OF CLEAR CELL RCC:

- blood
- large cohesive cell groups
- abundant wispy cytoplasm with ill-defined edges
- cytoplasmic vacuoles
- large, round, eccentrically placed nucleus
- nucleoli vary in size depending on Fuhrman grade

Aspirates from a clear cell RCC are often quite bloody. Smears can be highly cellular, but in fact sometimes reveal only blood; in such cases tissue fragments are often recovered in cell block sections. With cellular smears, the tumor cells are displayed as large tissue fragments and isolated cells. The malignant cells have abundant cytoplasm and thus a low nuclear-to-cytoplasmic ratio, with a round to slightly irregular, eccentrically placed nucleus^{15,17,24,114} (Fig. 14.8A, B). The eccentrically positioned, round nucleus gives the cells a plasmacytoid appearance (Fig. 14.8C); the nucleus is sometimes so far off-center that it appears partially extruded. The size of the nucleolus depends on the grade of the tumor:

small and inconspicuous in grade 1 tumors, progressively more prominent in grade 2, 3, and 4 tumors. The cytoplasm is thin and wispy, and the cell membranes are poorly defined. Small cytoplasmic vacuoles are often peripherally placed, whereas the remaining, more granular, cytoplasm is central. Higher grade tumors have more isolated cells, less cytoplasmic vacuolization, and, as mentioned, larger nucleoli (Fig. 14.8D). Pink, strand-like fibrillary material, possibly basement membrane material, is seen with Romanowsky stains and is highly characteristic (see Fig. 14.8B). Cytologic preparations from roughly one half of RCCs demonstrate so-called “transgressing vessels”¹¹⁵; their diagnostic significance is unclear, however, because some other tumors show similar features. Low-grade tumors are challenging because fragments of the tumor are extremely cohesive; one encounters large, highly cellular aggregates in which the individual cells are difficult to identify. A careful search around the perimeter of these chunks reveals the characteristic cells. Grade 1 to 2 clear cell RCCs, sometimes a challenge to interpret correctly on direct smears, are often surprisingly straightforward on cell block sections, in which the clear cell morphology is especially easy to recognize (Fig. 14.8E).

DIFFERENTIAL DIAGNOSIS OF CLEAR CELL RENAL CELL CARCINOMA:

- normal distal tubular cells
- macrophages
- adrenal cortical cells
- hepatocytes
- cystic nephroma
- AML (epithelioid)
- papillary RCC

The differential diagnosis includes benign tubular cells, which rarely emerge as large groups and do not have vacuolated cytoplasm. Macrophages, as in xanthogranulomatous pyelonephritis, mimic the cells of RCC but are more dispersed rather than aggregated and more uniformly microvacuolated; also, they lack nuclear atypia and an extruded nucleus. Adrenal cortical cells are more uniform, have smaller vacuoles, and are frequently stripped of their cytoplasm. Hepatocytes have a more uniformly granular cytoplasm containing lipofuscin, and have round nuclei of various sizes that are almost always centrally placed. Specimens from a cystic nephroma are generally hypocellular, but sometimes contain occasional atypical cells with clear to vacuolated cytoplasm, nuclear membrane irregularity, and prominent nucleoli. An unequivocal diagnosis of RCC should be avoided if the sample is sparsely cellular, particularly if a cell block is unavailable. Some AMLs have an epithelioid morphology. Some of these were misdiagnosed in the past as RCCs, even after histopathologic examination of a nephrectomy specimen. It is advisable

to consider the diagnosis of an epithelioid AML whenever a kidney tumor has an epithelioid morphology; the diagnosis of an AML can be excluded by demonstrating negative staining for HMB45 and MART-1. Similarly, some high-grade papillary RCCs are virtually indistinguishable from a clear cell RCC by conventional morphology. Cytogenetic analysis can be particularly helpful³⁵ because clear cell RCC is characterized by deletion of 3p (see Fig. 14.8F, Table 14.1). The clear cell RCCs with predominantly granular cytoplasm can look like oncocytomas.

Papillary Renal Cell Carcinoma

Papillary RCC represents between 7% and 15% of all RCCs.^{45,116,117} This subtype of RCC is associated with trisomies of chromosomes 7, 16, and 17^{35,44,118,119}; renal cortical adenomas¹²⁰; and multifocality.¹²⁰ Tumors have measured

as large as 23 cm, and large tumors can be cystic and necrotic. Contrast-enhanced imaging studies reveal an avascular or hypovascular lesion,¹¹⁷ in contrast to clear cell RCCs, which are hypervascular. Patients with a low-grade or low-stage papillary RCC have an excellent prognosis,^{116,117,119} whereas those with high-grade or high-stage papillary RCC have a poor prognosis.^{119,121} Low-grade papillary RCCs are often small and peripheral and are amenable to partial nephrectomy. Despite the high rate of multifocality, patients do not appear to be at increased risk for local recurrence after partial nephrectomy.

An RCC is assigned the papillary subtype histologically if at least 50% of it is comprised of true papillae. In some cases the papillae are so closely packed that the architecture is obscured, and the cells appear to grow in solid sheets.⁴⁵ Papillary RCCs are further divided into two basic morphologic subtypes, types 1 and 2, which

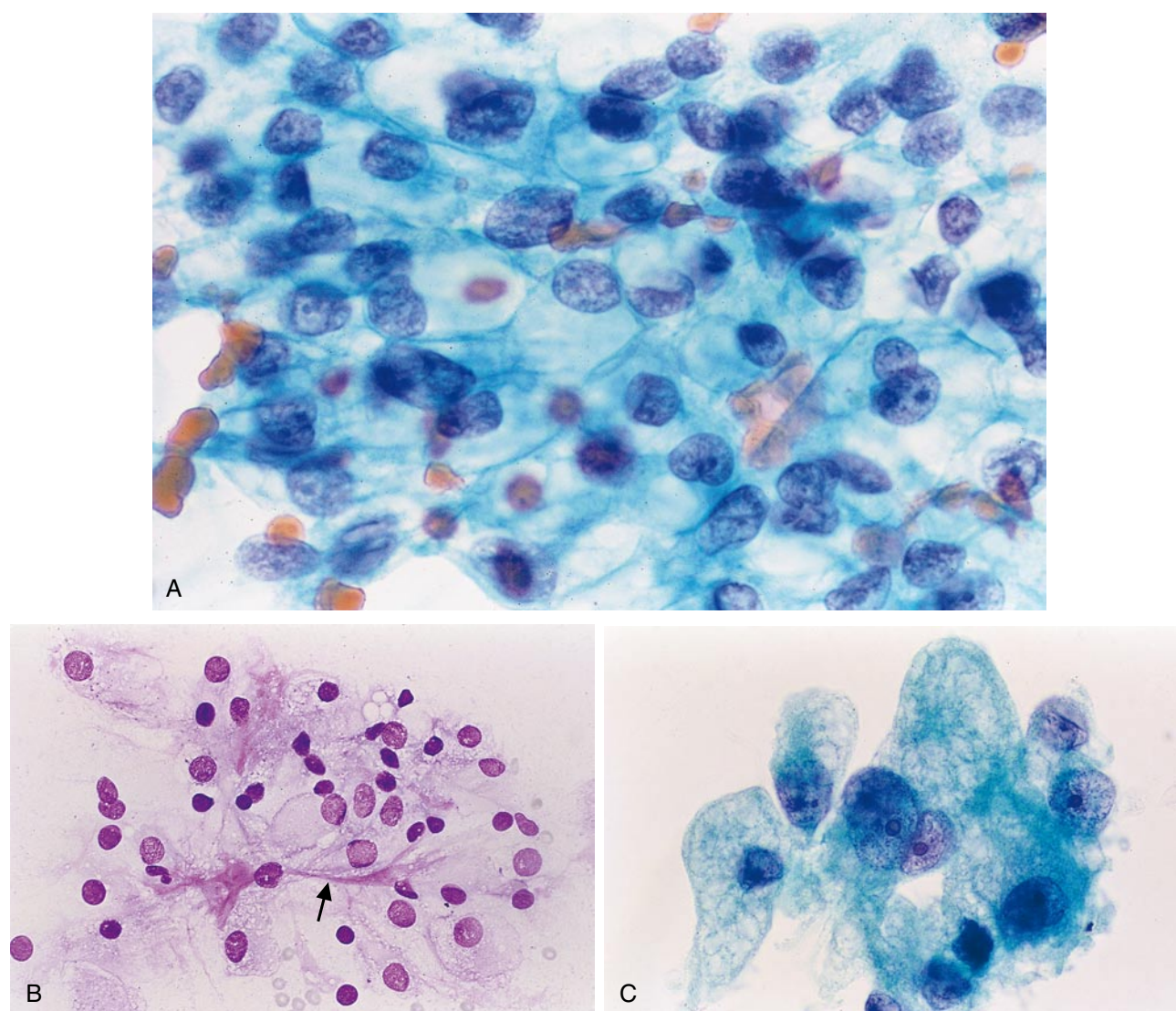


Figure 14.8 Clear cell renal cell carcinoma (RCC). A, The tumor cells are in a broad sheet and have abundant clear and granular cytoplasm (Papanicolaou stain). B, Fine cytoplasmic vacuoles and pink, strandlike material (arrow) are well seen with the Romanowsky stain. C, Nuclei are often eccentrically placed (Papanicolaou stain).

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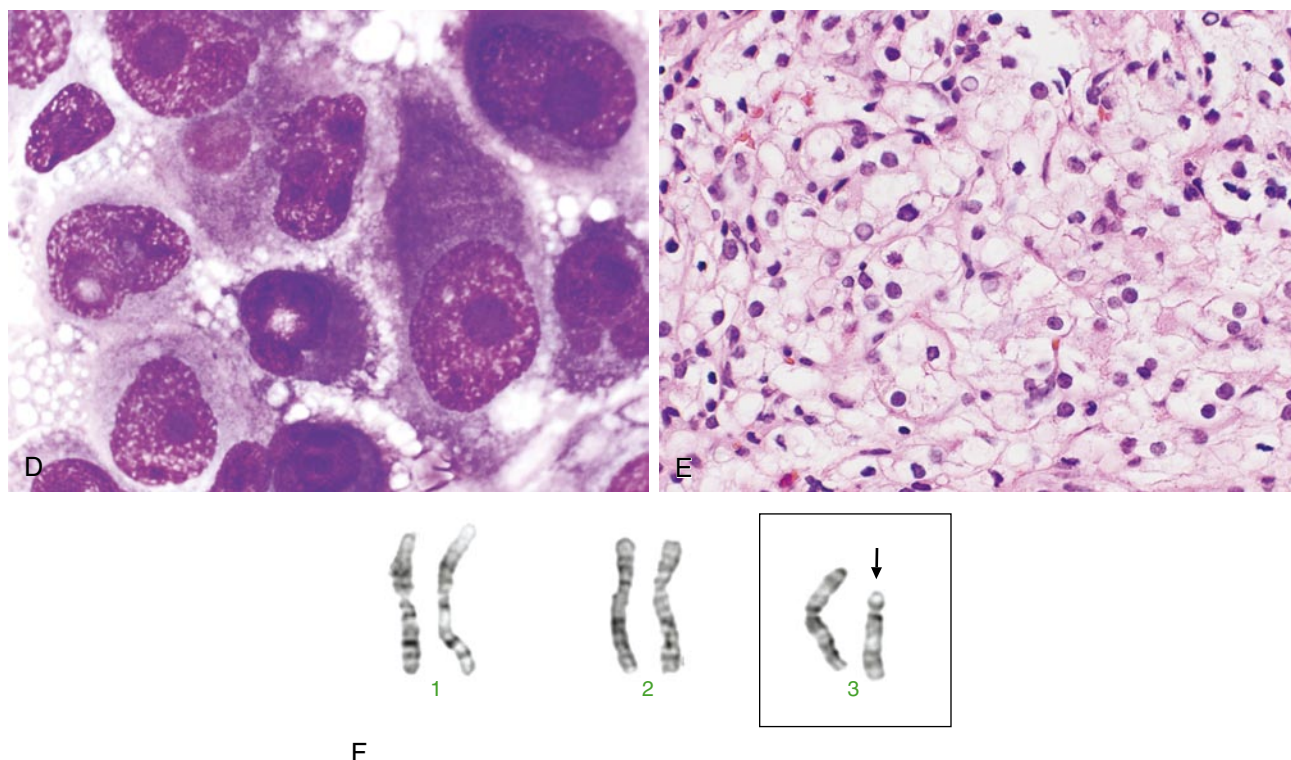


Figure 14.8 (Continued) **Clear cell renal cell carcinoma (RCC).** *D*, The cytoplasm of this grade 4 tumor is more opaque, the cells are more dyshesive, and nucleoli are prominent (Wright-Giemsa stain). *E*, Cell block sections reveal the characteristic clear cell morphology (hematoxylin-eosin [H & E] stain). *F*, A karyotype reveals the characteristic 3p deletion (arrow).

correlate with tumor grade. Thus, type 1, the more common type, is low-grade tumors composed of small cells with scant cytoplasm. Nuclei are small and round and nucleoli are inconspicuous (Fuhrman grade 1 or 2). Type 2 is less common, high-grade tumors composed of large cells with abundant granular cytoplasm. Nuclei of type 2 tumors are large, and nucleoli are prominent (grade 3). As can be seen from this description, type 2 papillary RCCs can resemble the higher-grade clear cell RCCs. Papillary RCCs are immunoreactive for EMA, low-molecular-weight keratins, and CK7,^{45,122} and negative for high-molecular-weight keratin 34BE12 and WT1.

CYTOMORPHOLOGY OF PAPILLARY RENAL CELL CARCINOMA (TWO TYPES):

Type 1 (low grade):

- papillae and spherules
- small-sized to medium-sized cuboidal cells
- uniform nuclei
- scant-to-moderate amount of cytoplasm
- abundant intracytoplasmic hemosiderin
- foamy macrophages

Type 2 (high grade):

- large cells
- large nuclei with grade 3 nucleoli
- abundant granular cytoplasm

Papillary RCCs have a number of characteristic cytologic features.¹²³⁻¹²⁶ These include papillae with true fibrovascular cores,¹¹⁵ spherules, and tubules (Fig. 14.9A). Fibrovascular cores distended with foamy macrophages are common and especially well seen on cell block preparations (Fig. 14.9B); indeed, the cell block is often the easiest way to correctly identify a papillary RCC. The tumor cells can be extremely uniform (Fig. 14.9C) when the tumor is type 1 (low-grade). In some cases there is abundant intracytoplasmic hemosiderin, a characteristic feature of papillary RCC (Fig. 14.9D). Psammoma bodies occur but only in a small minority of papillary RCCs.¹²³

DIFFERENTIAL DIAGNOSIS OF PAPILLARY RENAL CELL CARCINOMA:

- distal tubular cells
- glomeruli
- metanephric adenoma
- papillary hyperplasia in cysts associated with dialysis or adult polycystic kidney disease
- clear cell RCC

The cells of a low-grade (type 1) papillary RCC resemble benign distal tubular cells, but benign tubular cells are rarely present in large numbers, and never show papillae or spherule formation. Glomeruli, with their densely

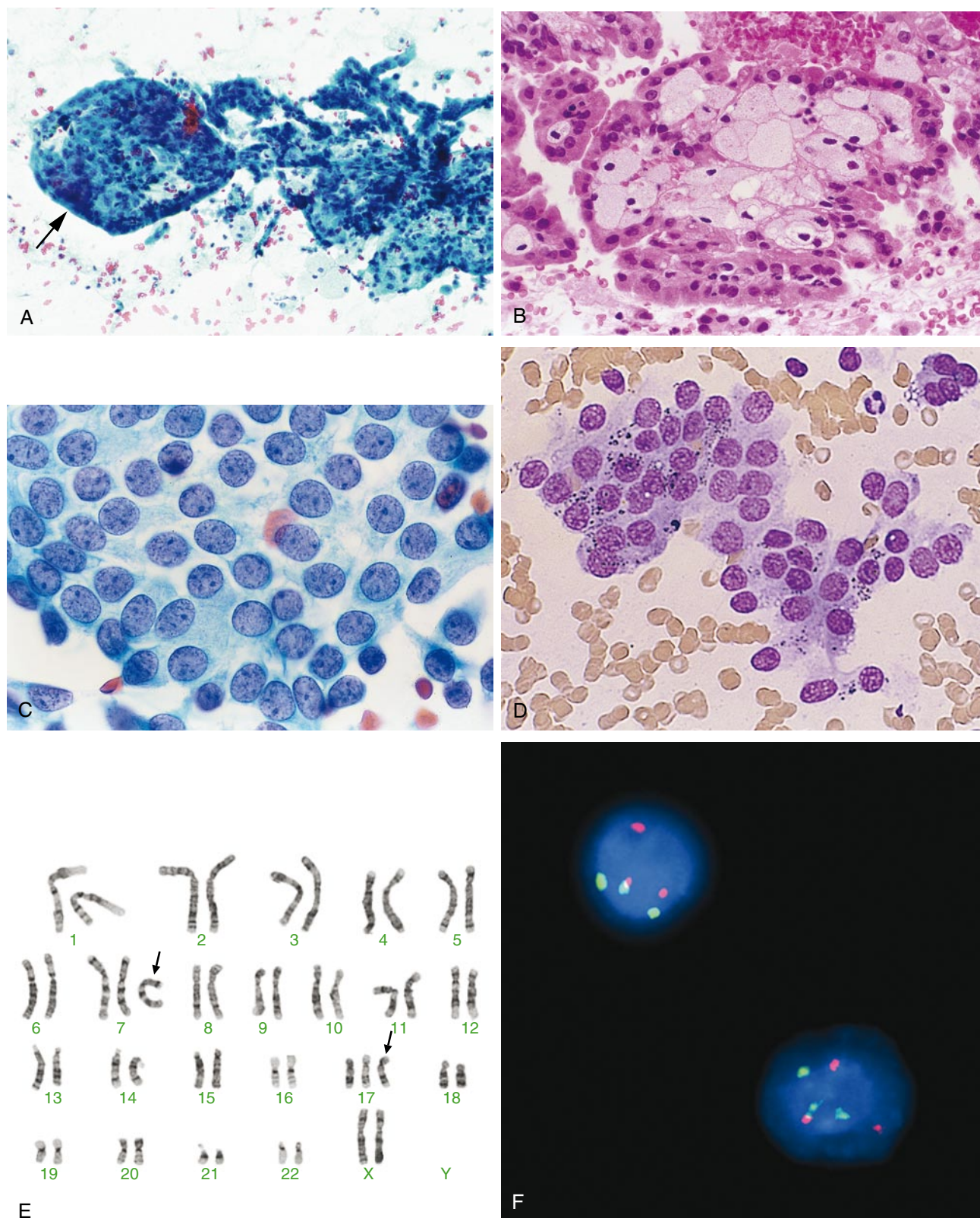


Figure 14.9 Papillary renal cell carcinoma (RCC). A, A complex papillary architecture is apparent on smears. Note the large sphere (arrow), a characteristic finding. Foamy macrophages are scattered in the background (Papanicolaou stain). B, Foamy macrophages stuffing the fibrovascular cores of the papillae are often best seen on cell block sections. (Hematoxylin-eosin [H & E] stain). C, Note how round and monomorphous the nuclei are in a low-grade papillary RCC (Papanicolaou stain). D, Cytoplasmic hemosiderin is a common finding (Wright-Giemsa stain). E, A karyotype reveals trisomies of chromosomes 7 and 17 (arrows). F, Fluorescence in situ hybridization (FISH) shows trisomies of chromosomes 7 (green signal) and 17 (red signal).

cellular, spherical arrangement, can be misinterpreted as a papillary RCC, but close observation reveals the capillary loops that identify them as glomeruli. Papillary hyperplasia occurs within the cysts of dialysis patients and those with adult polycystic disease, and distinction between this hyperplasia and carcinoma is not possible by FNA. (The distinction is controversial even histologically.) As previously mentioned, the best approach is to avoid aspirating cysts in patients on dialysis and those with adult polycystic kidney disease.

The differential diagnosis also includes clear cell RCC. Indeed, many high-grade (type 2) papillary RCCs are mistaken for clear cell RCCs.²³ Conventional cytogenetics or FISH can serve as an adjunct to cytology in distinguishing clear cell from papillary RCC based on their distinctive genetic aberrations (see Fig. 14.8E, F; Table 14.1).

Chromophobe Renal Cell Carcinoma

Chromophobe RCC is a distinctive variant that comprises 3% to 5% of all RCCs¹²⁷⁻¹²⁹ and is associated with numerous chromosomal monosomies.^{35,130-132} Patients have an excellent prognosis,^{129,133,134} unless their tumor is large or multifocal.¹³⁵

Histologically, the tumor is composed of trabeculae of cells with abundant, flocculent cytoplasm, distinct cell borders, frequent binucleation, dark chromatin, and irregular, “raisinoid” nuclear outlines. The cytoplasm is either pale (hence “chromophobic”; classic variant), eosinophilic (eosinophilic variant), or both (mixed variant). The cytoplasmic pallor of the classic variant is the result of the abundance of microvesicles, a characteristic ultrastructural feature.³³ The microvesicles push the remaining organelles to the periphery, so the pallor tends to be accentuated around the nucleus. Their prominent cell borders, characteristic nuclear changes, and cytoplasmic clearing make them resemble koilocytes, although the cytoplasmic clearing in chromophobe RCC is patchier, less sharply defined than it is in koilocytes. With HCl stain, the cytoplasm stains blue in a reticular (mesh-like) or coarse, droplet-like pattern.⁴¹

CYTOMORPHOLOGY OF CHROMOPHOBE RENAL CELL CARCINOMA:

- trabecular arrangement
- koilocytoid cells:
 - abundant cytoplasm
 - well-defined cell borders
 - binucleation
 - nuclear size variation
 - hyperchromatic chromatin without nucleoli
 - mitoses

Aspirates are highly cellular; in general, the cells are less cohesive than those of clear cell RCCs.¹³⁶⁻¹³⁸ The

abundant, irregularly granular, fluffy cytoplasm is characteristic (Fig. 14.10a). Nuclei show significant variation in size, occasional binucleation, hyperchromasia, irregular outlines and grooves, and pseudoinclusions.¹³⁹ With Romanowsky-stained smears, the perinuclear “reticulated” or vacuolated zone is particularly well seen (Fig. 14.10B). When fully developed and widespread, the koilocytoid appearance is unique to chromophobe RCCs.

DIFFERENTIAL DIAGNOSIS OF CHROMOPHOBE RENAL CELL CARCINOMA:

- clear cell or conventional RCC
- oncocytoma

The classic variant of chromophobe RCC is easily confused with a clear cell RCC, and the eosinophilic variant with an oncocytoma. In comparison to clear cell RCCs, chromophobe RCCs have more variation in cell and nuclear size, darker chromatin without nucleoli, and much more irregular nuclear outlines. Compared with oncocytoma, chromophobe tumors have more nuclear size variation and nuclear outline irregularity, but in some cases the distinction is impossible by routine cytologic stains alone. A tissue fragment in the cell block sections can be helpful. The cells of oncocytomas are arranged in rounded nests; one can draw a circle around groups of cells (see Fig. 14.3B), whereas the cells of a chromophobe RCC are arranged in endless trabeculae that are impossible to circumscribe (see Fig. 14.10C). In oncocytomas, there is more stroma between the nests of cells, whereas the trabeculae of a chromophobe RCC are so closely packed that there is little room for intervening stroma. Diffuse reticular or focal coarse, droplet-like cytoplasmic staining with HCl is characteristic of chromophobe RCC (Fig. 14.10D). This contrasts with the negative or focal apical staining of oncocytomas (Fig. 14.3C). Immunohistochemistry might also be helpful, particularly as experience in routine practice with proposed markers accrues.⁴² Fortunately, in the appropriate clinical setting, a partial nephrectomy is the preferred treatment for both lesions. In equivocal cases, a reasonable approach is to defer the distinction between oncocytoma and chromophobe RCC to extensive histologic sampling of the resected tumor, and interpret the FNA specimen as “Oncocytic Neoplasm (oncocytoma versus chromophobe RCC),” with a comment that a partial nephrectomy should be considered if clinically appropriate.

Sarcomatoid Renal Cell Carcinoma

Sarcomatoid RCC comprises 3% of all RCCs. It is a high-grade tumor with a poor prognosis: many are unresectable at presentation, and therefore candidates for FNA,^{140,141} although more and more are being identified

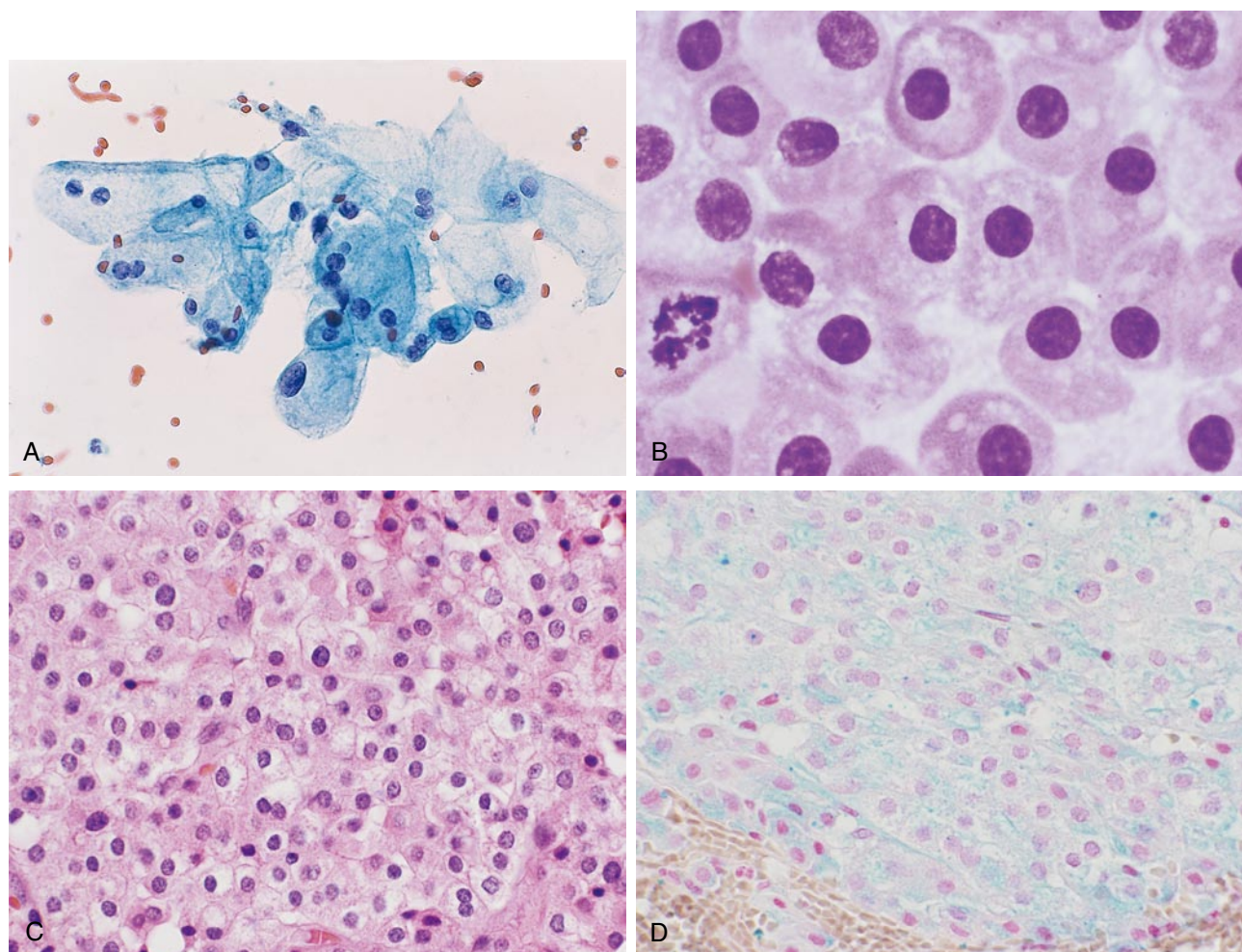


Figure 14.10 Chromophobe renal cell carcinoma (RCC), typical variant. A, The tumor cells have a koilocytopoid appearance, with abundant cytoplasm, prominent cell borders, binucleation, and dark nuclei that vary in size. Nucleoli are hard to see (Papanicolaou stain). B, The fluffy, irregularly vacuolated cytoplasm has a moth-eaten appearance (Wright-Giemsa stain). C, In cell block sections, the tumor cells are arranged in an endless sheet (hematoxylin-eosin [H & E] stain). D, The Hale's colloidal iron (HCl) stain shows a diffuse, reticulated staining pattern.

at a lower stage. Most are found in association with clear cell RCC, but sarcomatoid transformation of chromophobe, collecting duct, and papillary RCCs also occurs.

Histologically and cytologically, sarcomatoid RCC is a high-grade spindle cell neoplasm, with or without epithelioid differentiation^{114,142} (Fig. 14.11). If an epithelioid component is not identified, a sarcomatoid tumor must be immunoreactive for keratin proteins or EMA to merit a diagnosis of RCC. The differential diagnosis depends on sampling. If only the spindle cell area is sampled, a sarcoma and an AML are considered; if only the epithelioid area is sampled, the tumor is incorrectly classified as a clear cell RCC.

Collecting Duct Carcinoma (Bellini Tumor)

Collecting duct carcinoma is a rare, poorly defined tumor characterized by its medullary location, tubulopapillary histology, high-grade cytology, and prominent

desmoplasia.¹⁴³⁻¹⁵³ It resembles other tumors, especially renal medullary carcinoma¹⁵⁴ and papillary RCC.^{145,148,150,155,156} Characteristically, it is immunoreactive for high-molecular-weight cytokeratin 34BE12.^{143,149}

Cytologic features resemble those of a metastatic tumor: Cells have large, hyperchromatic nuclei and scant cytoplasm.¹⁵⁷⁻¹⁶¹

Translocation-Associated Renal Cell Carcinoma

Translocation-associated renal cell carcinoma was described in 1999 as a possible distinct histologic lesion that occurs primarily in children.¹⁶² Subsequent genetic studies confirmed the histologic features, identified cases in adults, and showed a strong association with translocations on chromosome X involving the transcription factor E3 (TFE3) gene.^{163,164} Histologically, the tumor has a mixed nested and papillary architecture made up of cells with

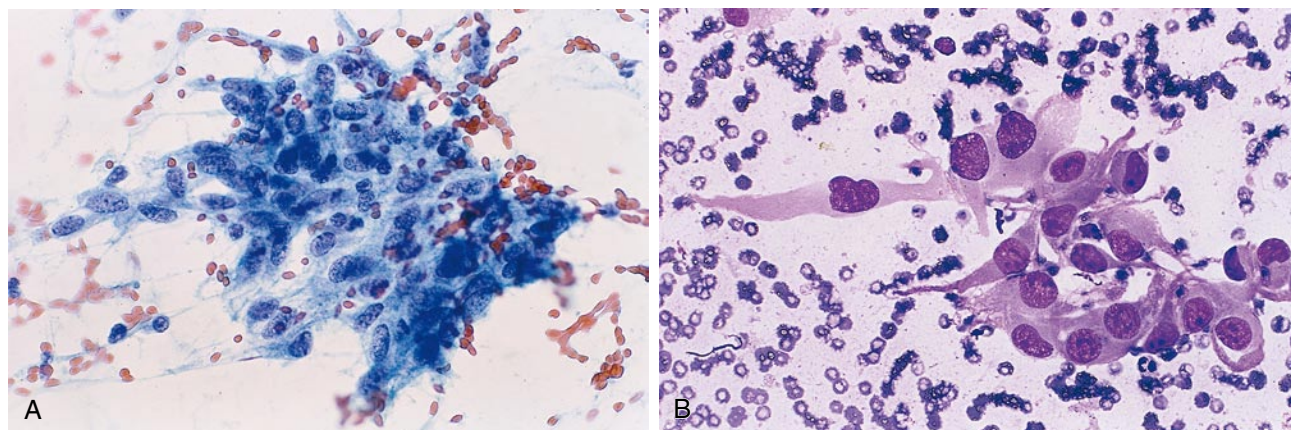


Figure 14.11 Sarcomatoid renal cell carcinoma (RCC). The malignant cells are haphazardly arranged and elongated. A, Papanicolaou stain. B, Wright-Giemsa stain.

abundant voluminous cytoplasm and frequent calcifications.¹⁶² The diagnosis is confirmed by demonstrating immunoreactivity for TFE3 and relatively scant staining for epithelial membrane antigen and keratin. Cytologic preparations reveal cells with abundant clear and granular cytoplasm.^{165,166} A strong clinical suspicion (young patient) and immunocytochemistry are necessary to distinguish this tumor from clear cell and other RCCs.

Urothelial Carcinoma

Urothelial carcinoma (UC) of the kidney, a malignancy that arises from the urothelium of the renal pelvis, is histologically and clinically similar to its bladder counterparts. It accounts for 5% to 10% of all renal tumors, and multifocality is common. There is a strong association with tobacco smoking and long-term use of analgesics, especially phenacetin. Other risk factors include UTIs, urinary stones, papillary necrosis, Balkan nephropathy, and radiologic contrast material that contains thorium. The most common presenting signs or symptoms are hematuria and flank pain.

Distinguishing UCs from RCCs is important because the management of a patient with a UC of the kidney requires resection of the ureter along with the kidney. The distinction between a UC and an RCC can sometimes be made based on imaging features, but this is more difficult with large tumors.

CYTOMORPHOLOGY OF UROTHELIAL CARCINOMA:

- large cells
- dark nuclei
- dense, smooth cytoplasm
- elongated cells
- cercariform cells

The cytologic appearance depends on the grade of the UC.^{11,13,15,167} Smears from low-grade tumors contain

sheets, papillae, and isolated cells with a moderate or abundant amount of smooth cytoplasm without vacuolization. In many cases, elongated cells with long cytoplasmic tails are prominent. Some of the tails are narrow in the middle and wide and flat at the end. These so-called cercariform (tadpole-like) cells¹⁶⁸⁻¹⁷⁰ are characteristic of UCs, and when present in large numbers, are helpful in distinguishing UC from tumors of other sites (Fig. 14.12). High-grade tumors are composed of isolated cells and small clusters with a scant to moderate amount of cytoplasm, dense hyperchromatic chromatin, and irregular nuclei. Cercariform cells may be present.

DIFFERENTIAL DIAGNOSIS OF UROTHELIAL CARCINOMA:

- RCC
- metastasis

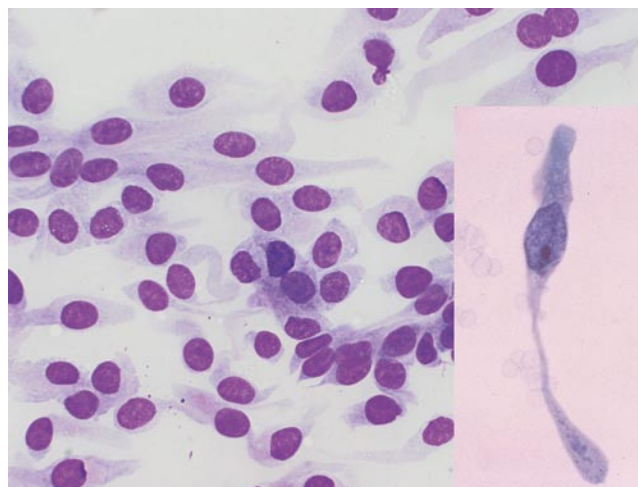


Figure 14.12 Urothelial carcinoma (UC). UCs typically contain numerous cells with elongated cytoplasm, some with flared ends called cercariform cells (Wright-Giemsa stain). *Inset:* Cercariform cell (Papanicolaou stain).

In difficult cases, immunocytochemical studies are helpful. UCs are variably positive for keratins 34BE12, CK20, CEA, and mucin; RCCs are negative for these markers. Conversely, RCCs are positive for CD10, RCCma, and PAX-2, whereas UCs are not. Distinction between UC and a metastasis can be difficult, but a history of a primary elsewhere; immunostaining for TTF-1 (lung) or CDX-2 (colon); and cercariform cells may be of value.

Metastatic Tumors

Metastases to the kidney are present in 7% of all cancer patients at autopsy,¹⁷¹ but most are clinically silent.

It is extremely uncommon for a metastasis to the kidney to be the initial manifestation of malignancy. Most metastases without a known primary probably represent unusual primary renal tumors.¹⁷²

The lung is the most common primary site. Most cases show cells with dark nuclei and irregular nuclear outlines^{11,15,23,173-175} (Fig. 14.13), but some lung metastases have abundant clear cytoplasm and prominent nucleoli and thus mimic high-grade clear cell RCC. Metastases like these can only be recognized as such by knowledge of the clinical history and judicious application of immunocytochemistry. Many metastases from the lungs

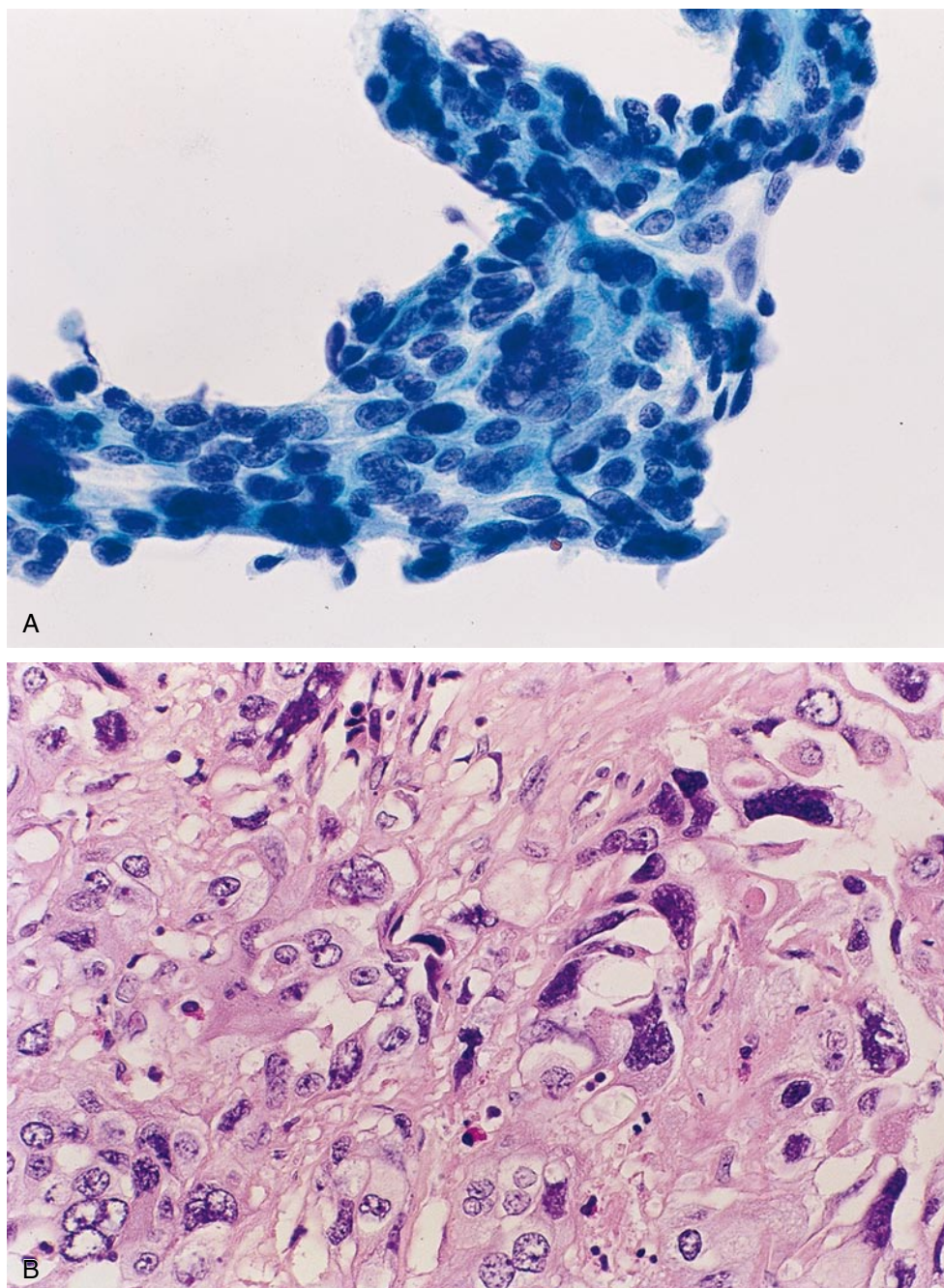


Figure 14.13 Metastatic lung cancer. These hyperchromatic, crowded malignant cells are not typical of renal cell carcinoma (RCC). In a patient with a history of lung cancer, the findings are most consistent with a metastasis from that site. A, Papanicolaou stain. B, hematoxylin-eosin (H & E) stained cell block.

are positive for mucin, CEA, TTF-1, and surfactant A. The distinction from typical variants of RCC is usually not difficult, particularly with immunocytochemistry because RCCs are often positive for CD10 renal cell carcinoma marker (RCCma), and PAX-2.¹⁷⁶ A variety of rare primary renal tumors, however, including collecting duct carcinoma, mucin-positive RCC,¹⁷⁷ small cell carcinoma of the kidney,¹⁷⁸ and primary lymphoma of the kidney,¹⁷⁹ are impossible to distinguish from a metastatic tumor by cytomorphology alone.

Primary renal tumors easily misinterpreted as a metastatic tumor:

- metanephric adenoma
- collecting duct carcinoma
- mucin-positive RCC
- primary small cell carcinoma of the kidney
- primary lymphoma of the kidney
- UC

Best diagnostic clue:

- clinical history (i.e., is there evidence of a primary tumor elsewhere?)

Although they are exceedingly rare, an unusual primary kidney tumor should be considered when one is confronted with the features previously described in a patient with no evidence of a malignancy elsewhere.

If there is a history of malignancy, comparison with the original tumor is often helpful in confirming that the renal tumor is in fact a metastasis (Fig. 14.14).

THE ADRENAL GLAND

The adrenal glands are a pair of triangular organs on top of the kidneys. They regulate metabolism by secreting cortisol; salt and water balance by secreting aldosterone; and response to stress by secreting a variety of hormones including epinephrine. Primary tumors of the adrenal gland fall into two principal categories: those that arise from the cortex (adrenal cortical adenoma and adrenal cortical carcinoma) and those that arise from the medulla (pheochromocytoma). Importantly, the adrenal gland is also a common site of metastasis from tumors elsewhere in the body. With the widespread use of CT, an increasing number of adrenal nodules are being detected, most commonly during a staging work-up for cancer elsewhere, but also for other complaints unrelated to the adrenal gland (adrenal incidentalomas).¹⁸⁰ FNA is an especially effective method for distinguishing benign adrenal nodules from metastatic tumors during staging procedures.¹⁸¹⁻⁸⁴ It is also useful for the diagnosis of some infectious diseases, such as disseminated histoplasmosis presenting as bilateral adrenal enlargement.¹⁸⁵ Its value in the assessment of an adrenal incidentaloma in a patient without a history of malignancy, however, is unclear.¹⁸⁶

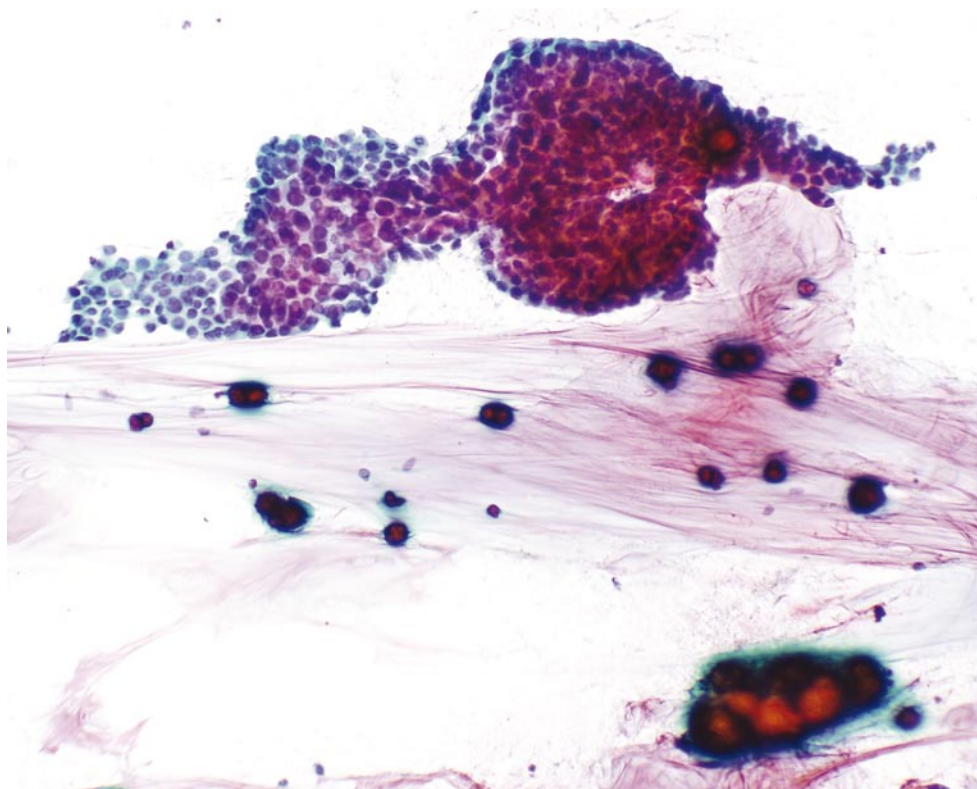


Figure 14.14 Metastatic breast cancer. There is abundant extracellular mucin, with clusters of malignant cells and occasional psammoma bodies. The resemblance to the patient's colloid carcinoma of the breast, resected 13 years earlier, was striking (Papanicolaou stain).

Specimen Collection, Preparation, and Accuracy

Virtually all adrenal aspirations are performed percutaneously by radiologists using CT or US imaging guidance.¹⁸⁰ A transhepatic approach is sometimes used, more commonly for right than for left adrenal nodules; in such cases, hepatocytes can be seen on FNA preparations. Complications are usually mild and include minimal hematuria and asymptomatic, self-limited hypotension and bradycardia. More serious complications are uncommon and include pneumothorax (2%) and hemothorax (1%) that may require a chest tube.¹⁸⁰ Fatal hemorrhage has been reported after aspiration of pheochromocytomas.¹⁸⁷

Slides are air-dried and stained with a Romanowsky stain, or alcohol-fixed and stained with the Papanicolaou or hematoxylin-eosin (H & E) stains. Cell blocks are helpful and provide an ideal platform for immunocytochemical studies

Adrenal FNA has an accuracy of 96% to 98%^{180,184} and good negative predictive value, particularly for lesions larger than 3 cm.¹⁸⁰ False-positive results are uncommon; some authors report no false-positives in their experience.¹⁸⁰ Rarely, the cells of a benign adrenal nodule or adenoma have been misinterpreted as metastatic small cell carcinoma.^{188,189} The nondiagnostic rate is 14%.^{180,190}

Myelolipoma

Myelolipoma, an uncommon benign neoplasm of the adrenal gland, is composed of adipose tissue and benign hematopoietic elements. Although myelolipomas are usually incidental findings, they vary considerably in size, and some are quite large; tumors larger than 30 cm in diameter have been reported. Aspirate material reveals fat with interspersed marrow elements including nucleated red blood cells, megakaryocytes, and granulocytes and their precursors.¹⁹¹ The differential diagnosis includes an AML of the kidney because both often contain fat, but the presence of hematopoietic elements combined with the absence of smooth muscle cells and negative immunoreactivity for HMB45 confirm the diagnosis.

Adrenal Cortical Neoplasms

Adrenal cortical adenomas are quite common, occurring in approximately 5% of adults, and their frequency increases with age. Adenomas are usually unilateral, solitary masses, in contrast to adrenal cortical hyperplasia, which is a bilateral, diffuse or multinodular enlargement. More than 85% of adenomas are non-functioning—they do not secrete cortisol, aldosterone, or other hormones. A minority are functioning adenomas, and the most frequent endocrine abnormalities associated with them are primary aldosteronism (Conn syndrome), Cushing

syndrome, virilization, and feminization. Histologically, there is no difference among the different types of functioning and nonfunctioning adenomas. Adenomas are composed of varying proportions of clear (lipid-filled) and granular cells, some with lipofuscin, arranged in cords and nests.

Adrenal cortical carcinomas are much less common than adenomas, with an incidence of one per million per year. In contrast to adenomas, 80% are functional; they can secrete cortisol, androgens, or a combination of both. They tend to be large tumors (>5 to 6 cm) that spread most commonly to the liver, lungs, retroperitoneum, and bones. Histologically, adrenal cortical carcinomas, like adenomas, are composed of a mix of clear and granular cells. Nuclear atypia can be minimal or pronounced. The mitotic rate varies widely. Several systems have been proposed for distinguishing adenomas from carcinomas histologically. They are multifactorial, and most include an evaluation of nuclear grade, necrosis, invasion, mitotic rate, and atypical mitoses.¹⁹²⁻¹⁹⁴ Because some of the features included in these systems cannot be assessed by FNA (e.g., capsular, sinusoidal invasion), they are impossible to apply rigorously to a cytologic specimen. Nevertheless, many adrenal tumors can be correctly classified as benign or malignant by FNA.

CYTOMORPHOLOGY OF BENIGN ADRENAL CORTICAL NODULE OR ADENOMA:

- numerous naked nuclei
- “frothy,” granular background
- occasional intact cells with bubbly cytoplasm

By imaging and cytomorphology it is not possible to distinguish an adrenal cortical adenoma from a hyperplastic nodule. For this reason, we refer to them henceforth as benign adrenal cortical nodules or adenomas. Benign adrenal cortical nodules or adenomas yield moderately cellular smears that have a characteristic pattern.¹⁹⁵ Smears show numerous isolated naked nuclei dispersed in a bubbly and granular background (Fig. 14.15A). Some overlapping nuclei show slight molding (Fig. 14.15B). There may be some variation in nuclear size, with occasional large nuclei, but mitoses are usually absent. Occasional cells with intact, bubbly (microvesicular) cytoplasm can be identified (Fig. 14.15C). Most cases contain large tissue fragments with benign spindled endothelial cells.¹⁹⁵ Some adenomas contain abundant intracellular lipofuscin pigment. Hepatocytes sometimes contaminate an adrenal FNA specimen¹⁹⁰ and can be confused with the cells of a benign adrenal cortical nodule or adenoma.¹⁹⁶ Both can have lipofuscin pigment, but hepatocytes have uniformly granular cytoplasm as opposed to the microvesicular cytoplasm of most adrenal cortical cells.

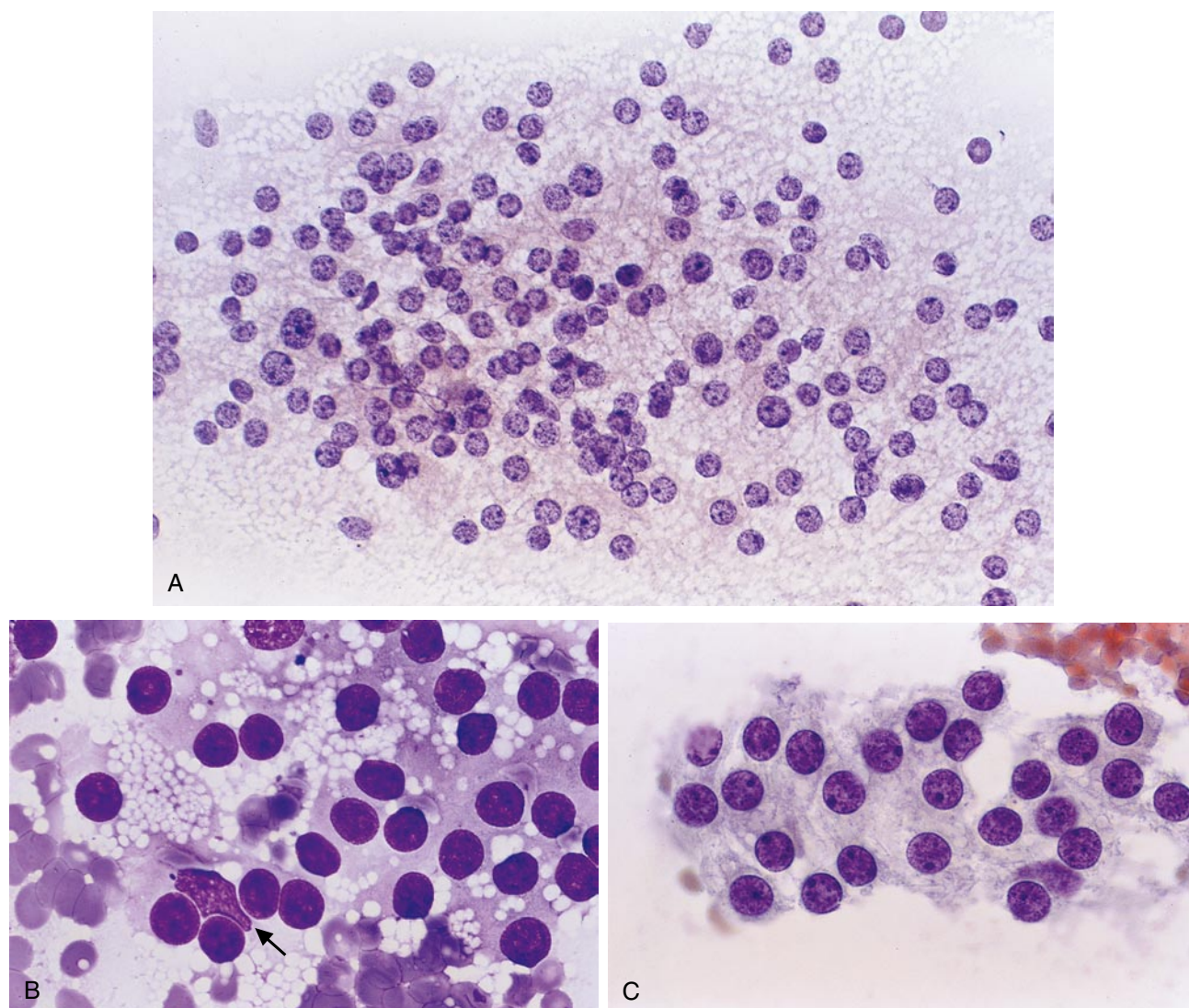


Figure 14.15 Benign adrenal cortical nodule or adenoma. A, Nuclei are stripped of cytoplasm and float in a sea of frothy cytoplasmic remnants (Papanicolaou stain). B, Adjacent nuclei occasionally show molding (arrow) and thus mimic small cell carcinoma (Wright-Giemsa stain). C, Intact cells have indistinct cell borders (Papanicolaou stain).

CYTOMORPHOLOGY OF ADRENAL CORTICAL CARCINOMA:

- numerous isolated cells with intact cytoplasm
- moderate to marked nuclear atypia
- mitoses
- necrotic debris

Adrenal cortical carcinomas range from well-differentiated to poorly differentiated tumors. In general, the pattern of isolated naked nuclei that is characteristic of benign adrenal cortical nodules or adenomas is not seen in carcinomas. Instead, the smears show numerous isolated cells with intact cytoplasm that is granular or vacuolated (Fig. 14.16A). Nuclei are enlarged and pleomorphic, especially when the tumor is moderately or poorly

differentiated, and mitoses can be seen (Fig. 14.16B). Necrotic debris may be present in the background.

Small (< 5 cm) tumors without atypia, necrosis, or mitoses pose no problems in diagnosis. Neither do large tumors with pleomorphism, abundant mitoses, atypical mitoses, necrosis, or clinical evidence of metastases. Distinguishing between a benign adrenal cortical nodule or adenoma and carcinoma can be perplexing, however, in tumors confined to the adrenal gland that show only mild or moderate nuclear atypia. When the findings are ambiguous, the interpretation “Adrenal cortical neoplasm of uncertain malignant potential” is reasonable.¹⁹⁰ Surgical excision is advised for precise classification of such nodules.

The differential diagnosis includes metastatic tumors.¹⁹⁵ Immunohistochemistry for inhibin melanA, and calretinin can be helpful.¹⁹⁸

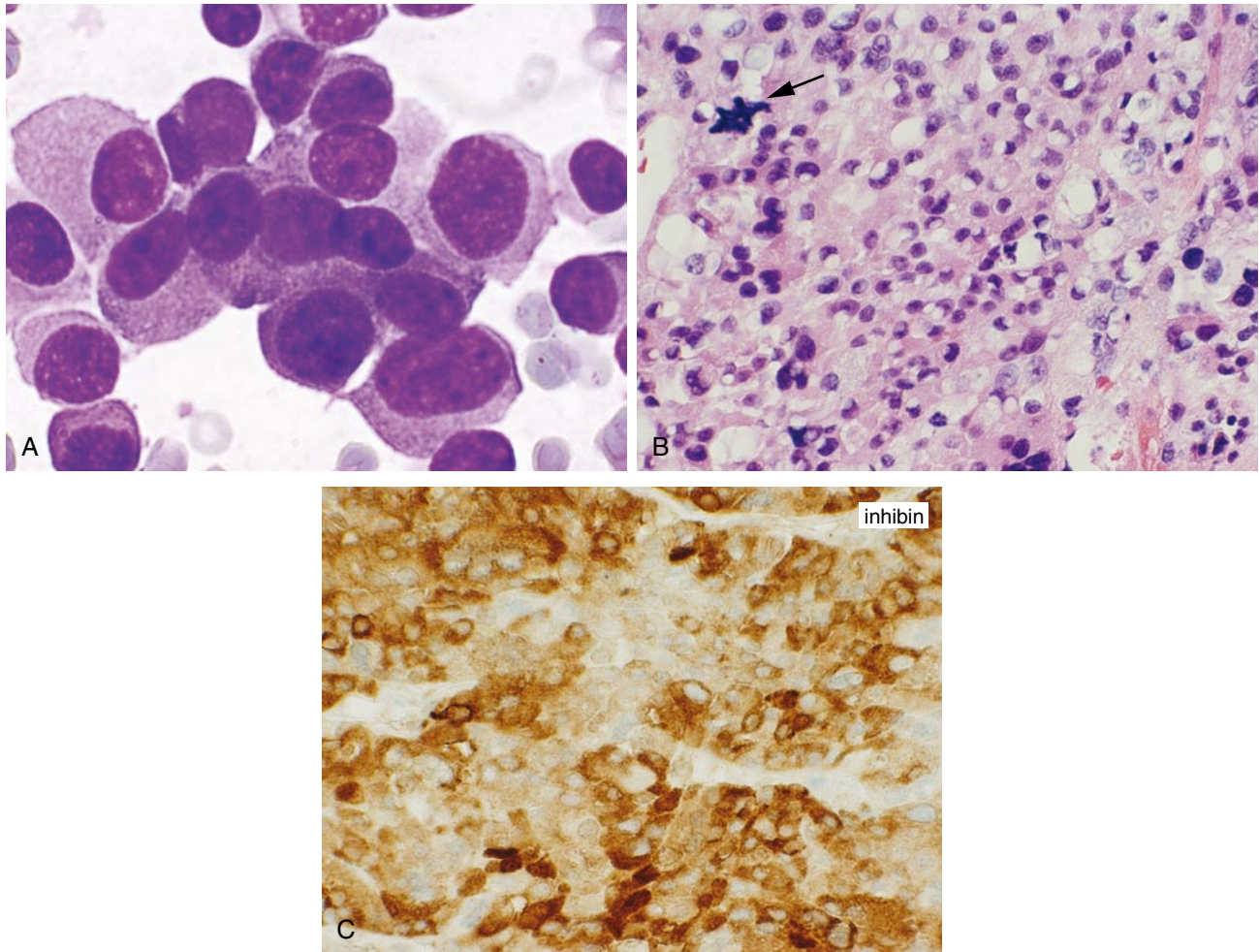


Figure 14.16 Adrenal cortical carcinoma. A, The malignant cells are large and have intact cytoplasm (Wright-Giemsa stain). B, Many tumors are highly pleomorphic and contain atypical mitoses (arrow). The mixture of clear and granular cytoplasm resembles that of a clear cell renal cell carcinoma (RCC); hematoxylin-eosin [H & E] stained cell block). C, Adrenal cortical carcinomas show cytoplasmic immunoreactivity for inhibin.

Pheochromocytoma

Pheochromocytomas arise from cells of the adrenal medulla and can cause paroxysmal hypertension as a result of excessive catecholamine production. In 10% to 20% of cases, they are associated with familial neoplastic syndromes, such as the multiple endocrine neoplasia syndromes 2a and 2b (MEN2), neurofibromatosis, and the VHL syndrome. A presumptive diagnosis of pheochromocytoma is based on the combination of an adrenal mass, hypertension, and elevated blood and urinary levels of catecholamines and their metabolites such as vanillylmandelic acid. From 10% to 20% of tumors are bilateral, most often in familial cases. Although 97% arise in the adrenal gland, a small percentage occur elsewhere, such as in the organs of Zuckerkandl; extra-adrenal pheochromocytomas are called paragangliomas.

Because episodic hypertension, hemorrhage, and even death have occurred with FNA, the procedure is generally avoided when a tumor is suspected of being a pheochromocytoma.¹⁹⁷

Cytologic preparations are highly cellular and contain cells arranged in loose clusters and as isolated cells (Fig. 14.17). Cellular pleomorphism can be marked, with small polygonal cells admixed with large spindle-shaped cells. Nuclei are pleomorphic and irregular in contour. The chromatin is finely stippled; intranuclear cytoplasmic pseudoinclusions and prominent nucleoli are present. Romanowsky-type stains show red cytoplasmic granules. With alcohol-fixed, Pap-stained preparations, the cytoplasm has a characteristic, deep red-to-purple, granular appearance.

Pheochromocytoma can resemble an adrenal cortical neoplasm. Immunostains assist in making the identification: pheochromocytomas are positive for chromo-

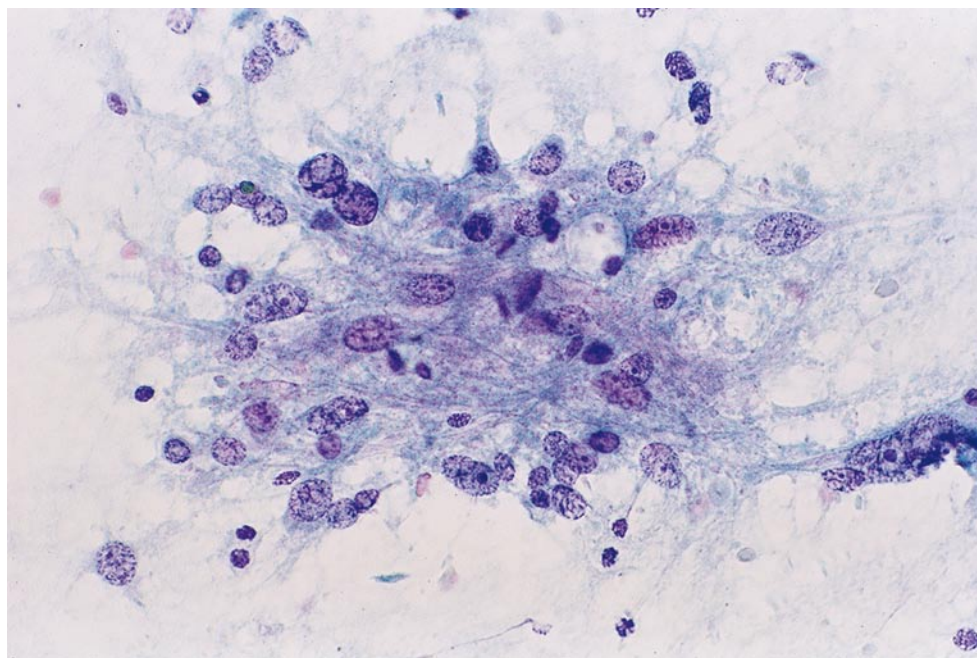


Figure 14.17 Pheochromocytoma. The elongated cells have abundant fibrillary cytoplasm (Papanicolaou stain).

granin and synaptophysin; adrenal cortical tumors are positive for inhibin, MelanA, and calretinin.¹⁹⁸

Metastatic Tumors

FNA of the adrenal gland truly shines when, with minimal invasiveness, it is used to confirm or rule out an adrenal metastasis in a patient with a history of cancer. The most common metastases encountered by FNA are from lung cancers, melanoma, and RCC.^{190,199} Certain tumors, like lung cancer and RCC, have a predilection for producing a solitary adrenal metastasis that mimics a primary adrenal lesion by imaging. In this setting, FNA plays a key role in patient management because it allows, in most cases, the distinction between a primary adrenal lesion (usually a benign adrenal cortical nodule or adenoma) and a solitary metastasis.

Comparison of cytologic or histologic material from the primary tumor, if available, to the FNA can be particularly helpful in establishing whether or not a nodule is a metastasis. Immunostains are helpful (e.g., TTF-1 for metastatic adenocarcinoma or small cell carcinoma of the lung; S-100 and HMB-45 for melanoma). One pitfall is worth pointing out: The dispersed, focally molded nuclei of a benign adrenal cortical nodule or adenoma resemble the cells of a small cell carcinoma of the lung^{188,189} (see Fig. 14.15B). The bubbly background and the absence of mitoses, necrosis, and elongated cells confirm that the lesion is a benign adrenal cortical nodule or adenoma and not metastatic small cell carcinoma.

Metastatic adenocarcinomas from the lung, kidney, breast, and other sites can look like an adrenal cortical carcinoma. Stains for mucin, CEA, keratin, and EMA, which are negative in adrenal cortical carcinomas, are usually positive in a metastatic adenocarcinoma. Adrenal cortical carcinoma can be particularly difficult to distinguish from a solitary metastasis of RCC. Immunostains for inhibin, A103/MelanA, and calretinin are helpful: adrenal cortical tumors are positive whereas RCCs are negative.^{198,200-204} Conversely, RCCs are positive for EMA, keratins, CD10, PAX-2, and RCCma, and adrenal cortical tumors are negative or only weakly positive for low-molecular-weight keratins.²⁰⁵

Some metastatic malignancies mimic a pheochromocytoma. The latter can be identified by its immunoreactivity for keratin proteins (if metastatic carcinoma), S-100 and HMB-45 (if melanoma),

Finally, the adrenal glands can be involved by Hodgkin and non-Hodgkin lymphomas.^{190,195,206} Adrenal involvement is usually found in the context of widespread disease, but rare examples of primary lymphoma of the adrenal have been reported. A rare and unusual lymphoma, the intravascular large B-cell lymphoma, has a predilection for involving the adrenal glands at presentation. It is an uncommon subtype of extranodal diffuse large B-cell lymphoma characterized by lymphoma cells confined to the lumina of blood vessels. This can result in significant enlargement, detectable by CT, of one or both adrenal glands. Morphologically, the malignant cells are indistinguishable from a diffuse large B-cell lymphoma.

Summary and helpful hints for the kidney and adrenal gland

- FNA is useful for the diagnosis of selected kidney masses.
- Thirty percent of renal aspirates are non-diagnostic.
- Never call a renal FNA specimen positive based on a small number of atypical cells; it is likely the kidney will be resected even if you report the results as suspicious. Benign lesions can have rare highly atypical cells.
- FNA has low sensitivity for the diagnosis of a cystic RCC.
- RCCs can and should be subclassified by FNA.
- Both oncocytoma and chromophobe RCC are candidates for partial nephrectomy; you may not always be able to distinguish them.
- Renal adenoma is not a diagnostic consideration when evaluating an FNA from a radiographically visible mass.
- The FNA diagnosis of an AML is challenging because only the radiographically indeterminate masses (i.e., those with low fat content) are selected for FNA.
- Metanephric adenomas are benign and negative for EMA, and they may be difficult to distinguish from a Wilms tumor.
- UC cells often have tails (cercariform cells) and are frequently immunoreactive for CEA and CK20.
- The cells of most metastases to the kidney have scant cytoplasm and dark, dense chromatin.
- Avoid diagnosing a metastasis to the kidney if there is no evidence for a primary elsewhere.
- Adrenal cortical lesions are immunoreactive for inhibin, A103/MelanA, and calretinin.

REFERENCES

1. Kelley CM, Cohen MB, Raab SS: Utility of fine-needle aspiration biopsy in solid renal masses. *Diagn Cytopathol* 1996;14:14-19.
2. Iyer VK, Agarwala S, Verma K: Fine-needle aspiration cytology of clear-cell carcinoma of the kidney: Study of eight cases. *Diagn Cytopathol* 2005;33:83-89.
3. Radhika S, Bakshi A, Rajwanshi A, et al.: Cytopathology of uncommon malignant renal neoplasms in the pediatric age group. *Diagn Cytopathol* 2005;32:281-286.
4. Tuncali K, vanSonnenberg E, Shankar S, et al: Evaluation of patients referred for percutaneous ablation of renal tumors: Importance of a preprocedural diagnosis. *AJR Am J Roentgenol* 2004;183(3):575-582.
5. Silverman SG, Gan YU, Morteale KJ, et al.: Renal masses in the adult patient: The role of percutaneous biopsy. *Radiology* 2006;240(1):6-22.
6. Renshaw AA, Corless CL: Papillary renal cell carcinoma: gross features and histologic correlates. *J Urol Pathol* 1997;7:9-19.
7. Bossuyt V, Ginevan K, dal Cin P, et al.: The value of cytogenetics as an adjunct to cytology in the diagnosis of non-hematopoietic neoplasms. *Mod Pathol* 2007;20(Suppl. 2):64A.
8. Brierly RD, Thomas PJ, Harrison NW, et al.: Evaluation of fine-needle aspiration cytology for renal masses. *BJU Int* 2000;85:14-18.
9. Cristallini EG, Paganelli C, Bolis GB: Role of fine-needle aspiration biopsy in the assessment of renal masses. *Diagn Cytopathol* 1991;7:32-35.
10. Dekmezian RH, Charnsangavej C, Rava P, Katz RL: Fine needle aspiration of kidney tumors in 105 patients: A cytologic and histologic correlation (Abstract). *Acta Cytol* 1985;29:931.
11. Helm CW, Burwood RJ, Harrison NW, Melcher DH: Aspiration cytology of solid renal tumors. *J Urol* 1983;55:249-253.
12. Johnson PT, Nazarian LN, Feld RI, et al.: Sonographically guided renal mass biopsy: Indications and efficacy. *J Ultrasound Med* 2001;20:749-753.
13. Juul N, Torp-Pedersen S, Gronvall S, et al.: Ultrasonically guided fine needle aspiration biopsy of renal masses. *J Urol* 1985;133:579-581.
14. Lechevallier E, Andre M, Barriol D, et al.: Fine-needle percutaneous biopsy of renal masses with helical CT guidance. *Cancer* 2000;216:506-510.
15. Murphy WM, Zambroni BR, Emerson LD, et al.: Aspiration biopsy of the kidney. *Cancer* 1985;56:200-205.
16. Neuzillet Y, Lechevallier E, Andre M, et al.: Accuracy and clinical role of fine needle percutaneous biopsy with computerized tomography guidance of small (less than 4.0cm) renal masses. *J Urol* 2004;171:1802-1805.
17. Nguyen GK: Percutaneous fine-needle aspiration biopsy cytology of the kidney and adrenal. *Pathol Annu* 1987;22:163-191.
18. Pilotti S, Rilke F, Alasio L, Garbagnati F: The role of fine needle aspiration in the assessment of renal masses. *Acta Cytol* 1988;32:1-10.
19. Sengupta S, Zincke H, Blute ML: The accuracy of 250 fine needle biopsies of renal tumors. *J Urol* 2005;174:2422.
20. Truong LD, Todd TD, Dhurandhar B, Ramzy I: Fine-needle aspiration of renal masses in adults: Analysis of results and diagnostic problems in 108 cases. *Diagn Cytopathol* 1999;20:339-349.
21. Wunderlich H, Hindermann W, Mustafa AMA, et al.: The accuracy of 250 fine needle biopsies of renal tumors. *J Urol* 2005;174:44-46.
22. Zardawi IM: Renal fine needle aspiration cytology. *Acta Cytol* 1999;43:184-190.
23. Renshaw AA, Lee KR, Madge R, Granter SR: Accuracy of fine needle aspiration in distinguishing subtypes of renal cell carcinoma. *Acta Cytol* 1997;41:987-994.
24. Linsk JA, Franzen S: Aspiration cytology of metastatic hypernephroma. *Acta Cytol* 1984;28:250-260.
25. Alanen KA, Ekfors TO, Lipasti JA, Nurmi MJ: Renal oncocytoma: the incidence of 18 surgical and 12 autopsy cases. *Histopathology* 1984;8:731-737.
26. Choi H, Almagro U, McManus J, et al.: Renal oncocytoma. A clinicopathologic study. *Cancer* 1983;51:1887-1896.
27. Klein M, Valensi Q: Proximal tubular adenomas of kidney with so called oncocytic features. *Cancer* 1976;38:906-914.
28. Lieber M, Tomera K, Farrow G: Renal oncocytoma. *J Urol* 1981;125:481-485.
29. Lieber MM: Renal oncocytoma: prognosis and treatment. *Eur Urol* 1990;18:17-21.
30. Merino M, LiVolsi V: Oncocytomas of the kidney. *Cancer* 1982;50:1852-1856.
31. Perez-Ordóñez B, Hamed G, Campbell S, et al.: Renal oncocytoma: A clinicopathologic study of 70 cases. *Am J Surg Pathol* 1997;21:871-883.
32. Fuhrman S, Lasky L, Limas C: Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol* 1982;6:655-663.

33. Tickoo SK, Lee MW, Eble JN, et al.: Ultrastructural observations on mitochondria and microvesicles in renal oncocytoma, chromophobe renal cell carcinoma, and eosinophilic variant of conventional (clear cell) renal cell carcinoma. *Am J Surg Pathol* 2000;24(9):1247-1256.
34. Amin MB, Crotty TB, Tickoo SK, Farrow GM: Renal oncocytoma: A reappraisal of morphologic features with clinicopathologic findings in 80 cases. *Am J Surg Pathol* 1997;21:1-12.
35. Mitelman F, Johansson B, Mertens F (eds): Mitelman Database of Chromosome Aberrations in Cancer 2007. Available at <http://cgap.nci.nih.gov/Chromosomes/Mitelman>.
36. Alanen KA, Tyrkko JES, Nurmi MJ: Aspiration biopsy cytology of renal oncocytoma. *Acta Cytol* 1985;29:859-862.
37. Cochand-Priollet B, Rothschild E, Chagnon S, et al.: Renal oncocytoma diagnosed by fine-needle aspiration cytology. *Br J Urol* 1988;61:534-544.
38. Liu J, Fanning CV: Can renal oncocytomas be distinguished from renal cell carcinoma on fine-needle aspiration specimens? A study of conventional smears in conjunction with ancillary studies. *Cancer* 2001;93:390-397.
39. Nguyen GK, Amy RW, Tsang S: Fine needle aspiration biopsy cytology of renal oncocytoma. *Acta Cytol* 1985;29:33-36.
40. Rodriguez CA, Buskop A, Johnson J, et al.: Renal oncocytoma. *Acta Cytol* 1980;24:355-359.
41. Tickoo SK, Amin MB, Zarbo RJ: Colloidal iron staining in renal epithelial neoplasms, including chromophobe renal cell carcinoma: emphasis on technique and patterns of staining. *Am J Surg Pathol* 1998;22(4):419-424.
42. Liu L, Qian J, Singh H, Meiers I, et al.: Immunohistochemical analysis of chromophobe renal cell carcinoma, renal oncocytoma, and clear cell carcinoma: An optimal and practical panel for differential diagnosis. *Arch Pathol Lab Med* 2007;131(8):1290-1297.
43. Adley BP, Schafernak KT, Yeldandi AV, et al.: Cytologic and histologic findings in multiple renal hybrid oncocytic tumors in a patient with Birt-Hogg-Dube syndrome: A case report. *Acta Cytol* 2006;50:584-548.
44. Corless CL, Aburatani H, Fletcher JA, et al.: Papillary renal cell carcinoma: Quantitation of chromosome 7 and 17 by FISH, analysis of chromosome 3p for LOH, and DNA ploidy. *Diagn Mol Pathol* 1996;5:53-64.
45. Renshaw AA, Corless CL: Papillary renal cell carcinoma: histology and immunohistochemistry. *Am J Surg Pathol* 1995;19:842-849.
46. Storkel S, Eble JN, Adlakha K, et al.: Classification of renal cell carcinoma: Workgroup No 1. Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). *Cancer* 1997;80:987-989.
47. Blute ML, Malek RS, Segura JW: Angiomyolipoma: Clinical metamorphosis and concepts for management. *J Urol* 1988;139:20-24.
48. Farrow GM, Harrison EG, Utz DC, Jones DR: Renal angiomyolipoma: a clinicopathologic study of 32 cases. *Cancer* 1968;22:564-570.
49. Murphy WM, Beckwith JB, Farrow GM: Tumors of the kidney, bladder, and related urinary structures, 3rd ed. Washington, DC: Armed Forces Institute of Pathology, 1994.
50. Cibas ES, Goss GA, Kulke MH, et al.: Malignant epithelioid angiomyolipoma ('sarcoma ex angiomyolipoma') of the kidney: a case report and review of the literature. *Am J Surg Pathol* 2001;25:121-126.
51. Crapanzano JP: Fine-needle aspiration of renal angiomyolipoma: cytological findings and diagnostic pitfalls in a series of five cases. *Diagn Cytopathol* 2005;32:53-57.
52. Glenthøj A, Partoft S: Ultrasound-guided percutaneous aspiration of renal angiomyolipoma. *Acta Cytol* 1984;28:265-268.
53. Gupta RK, Nowitz M, Wakefield SJ: Fine needle aspiration cytology of renal angiomyolipoma: Report of a case with immunocytochemical and electron microscopic findings. *Diagn Cytopathol* 1998;18:297-300.
54. Mai KT, Yazdi HM, Perkins DG, Thijssen A: Fine needle aspiration biopsy of epithelioid angiomyolipoma. A case report. *Acta Cytol* 2001;45:233-236.
55. Mojica WD, Jovanoska S, Bernacki EG: Epithelioid angiomyolipoma: Appearance on fine-needle aspiration report of a case. *Diagn Cytopathol* 2000;23:192-195.
56. Nguyen GK: Aspiration biopsy cytology of renal angiomyolipoma. *Acta Cytol* 1984;28:261-264.
57. Pancholi V, Munjal K, Jain M, et al.: Pre-operative diagnosis of a renal angiomyolipoma with fine needle aspiration cytology: A report of 3 cases. *Acta Cytol* 2006;50:466-468.
58. Sangawa A, Shintaku M, Nishimura M: Nuclear pseudoinclusions in angiomyolipoma of the kidney. A case report. *Acta Cytol* 1998;42:425-429.
59. Sant GR, Ayers DK, Bankoff MS, et al.: Fine needle aspiration biopsy in the diagnosis of renal angiomyolipoma. *J Urol* 1990;143:999-1001.
60. Taavitsainen M, Krogeris L, Rannikko S: Aspiration biopsy of renal angiomyolipoma. *Acta Radiol* 1989;30:381-382.
61. Wadih GE, Raab SS, Silverman JF: Fine needle aspiration cytology of renal and retroperitoneal angiomyolipoma. *Acta Cytol* 1995;39:945-950.
62. Miranda MC, Saigo PE: Fine needle aspiration biopsies of primary renal neoplasms (abstract). *Acta Cytol* 1996;40:1026.
63. Davis CJ, Barton JH, Sesterhenn IA, Mostofi FK: Metanephric adenoma. Clinicopathological study of fifty patients. *Am J Surg Pathol* 1995;19:1101-1114.
64. Gatalica Z, Grujic S, Kovatich A, Petersen RO: Metanephric adenoma: Histology, immunophenotype, cytogenetics, ultrastructure. *Mod Pathol* 1996;9:329-333.
65. Jones EC, Pins M, Dickersin GR, Young RH: Metanephric adenoma of the kidney. *Am J Surg Pathol* 1995;19:615-626.
66. Strong JW, Ro JY: Metanephric adenoma of the kidney: a newly characterized entity. *Adv Anat Pathol* 1996;3:172-178.
67. Renshaw AA, Freyer DR, Hammers YA: Metastatic metanephric adenoma in a child. *Am J Surg Pathol* 2000;24:570-574.
68. Renshaw AA, Maurici D, Fletcher JA: Cytologic and fluorescence in situ hybridization (FISH) examination of metanephric adenoma. *Diagn Cytopathol* 1997;16:107-111.
69. Argani P: Metanephric neoplasms: The hyperdifferentiated, benign end of the Wilms tumor spectrum. *Clin Lab Med* 2005;25:379-392.
70. Olgac S, Hutchinson B, Tickoo SK, Reuter VE: Alpha-methylacyl-CoA racemase as a marker in the differential diagnosis of metanephric adenoma. *Mod Pathol* 2006;19(2):218-224.
71. Sherman ME, Silverman JML, Balogh K, Tan SS: Multilocular renal cyst. *Arch Pathol Lab Med* 1987;111:732-736.
72. Taxy JB, Marshall FF: Multilocular renal cysts in adults. *Arch Pathol Lab Med* 1983;107:633-637.
73. Clark SP, Kung ITM, Tang SK: Fine-needle aspiration of cystic nephroma (multilocular cyst of the kidney). *Diagn Cytopathol* 1992;8:349-351.
74. Hughes JH, Niemann TH, Thomas PA: Multicystic nephroma: report of a case with fine-needle aspiration findings. *Diagn Cytopathol* 1996;14:60-63.
75. Morgan C, Greenberg ML: Multilocular renal cyst: A diagnostic pitfall on fine-needle aspiration cytology: Case report. *Diagn Cytopathol* 1995;13(1):66-70.
76. Koss LG: *Diagnostic Cytology of the Urinary Tract*. Philadelphia, Lippincott-Raven, 1996.
77. Silverman JE, Gurley AM, Harris JP, et al.: Fine needle aspiration cytology of renal infarcts. *Acta Cytol* 1991;35:736-741.

78. de Kernion JB, Belldegrun A: Renal tumors. In Walsh PC, Retik AB, Stamey TA, Vaughan ED (eds): *Campbells Urology*. Philadelphia, WB Saunders, 1992, pp. 1055-67.
79. Lang EK: Renal cyst puncture studies. *Urol Clin N Am* 1987;14:91-102.
80. Emmett JL, Levine SR, Woolner LB: Co-existence of renal cyst and tumour: Incidence in 1,007 cases. *Br J Urol* 1963;35:403-410.
81. Gibson TE: Inter-relationship of renal cysts and tumors: report of three cases. *J Urol* 1954;71:241-252.
82. Khorsand D: Carcinoma within solitary renal cysts. *J Urol* 1965;93:440-444.
83. Lang EK: Coexistence of cyst and tumor in the same kidney. *Diagn Radiol* 1971;101:7-16.
84. Levine SR, Emmett JL, Woolner LB: Cyst and tumor occurring in the same kidney. *J Urol* 1964;91:8-9.
85. Ljunberg B, Holmberg G, Sjodin JG, et al.: Renal cell carcinoma in a renal cyst: A case report and review of the literature. *J Urol* 1990;143:797-799.
86. Navari RM, Ploth DW, Tatum RK: Renal adenocarcinoma associated with multiple simple cysts. *JAMA* 1981;246:1808-1809.
87. Corica FA, Iczkowski KA, Cheng L, et al.: Cystic renal cell carcinoma is cured by resection: A study of 24 cases with long-term follow-up. *J Urol* 1999;161:408-411.
88. Suzigan S, Lopez-Beltran A, Montironi R, et al.: Multilocular cystic renal cell carcinoma: A report of 45 cases of a kidney tumor of low malignant potential. *Am J Clin Pathol* 2006;125:217-222.
89. Brunn E, Nielsen K: Solitary cyst and clear cell adenocarcinoma of the kidney: Report of 2 cases and review of the literature. *J Urol* 1986;136:449-451.
90. Aronson S, Frazier HA, Baluch JD, et al.: Cystic renal masses: Usefulness of the Bosniak classification. *Urol Radiol* 1991;13:83-90.
91. Aubert S, Zini L, Delomez J, et al.: Cystic renal cell carcinoma in adults: Is preoperative recognition of multilocular cystic renal cell carcinoma possible? *J Urol* 2005;174:2115-2119.
92. Bosniak MA: Difficulties in classifying cystic lesions of the kidney. *Urol Radiol* 1991;13:91-93.
93. Bosniak MA: The small (< 3.0cm) renal parenchymal tumor: Detection diagnosis and controversies. *Radiology* 1991;179:307-317.
94. Bosniak MA: Problems in the radiologic diagnosis of renal parenchymal tumors. *Urol Clin N Am* 1993;20:217-230.
95. Pollack HM, Banner MP, Arger PH, et al.: The accuracy of gray-scale renal ultrasonography in differentiating cystic neoplasms from benign cysts. *Radiology* 1982;143:741-745.
96. Ambrose SS, Lewis EL, Obrien DP, et al.: Unsuspected renal tumors associated with renal cysts. *J Urol* 1977;117:704-707.
97. Plowden KM, Erozan YS, Frost JK: Cellular atypias associated with benign lesions of the kidney as seen in fine needle aspirates (Abstract). *Acta Cytol* 1984;28:648-649.
98. Todd TD, Dhurandar B, Mody D, et al.: Fine-needle aspiration of cystic lesions of the kidney. Morphologic spectrum and diagnostic problems in 41 cases. *Am J Clin Pathol* 1999;111:317-328.
99. Lang EK: Roentgenographic assessment of asymptomatic renal lesions. *Radiology* 1973;109:257-269.
100. Noshier JL, Amorosa JK, Leiman S, Plafker J: Fine needle aspiration of the kidney and adrenal gland. *J Urol* 1982;128:895-899.
101. Kleist H, Jonsson O, Lundstam S, et al.: Quantitative lipid analysis in the differential diagnosis of cystic renal lesions. *Br J Urol* 1982;54:441-445.
102. Bretan PN, Busch MP, Hricak H, Williams RD: Chronic renal failure: a significant risk factor in the development of acquired renal cysts and renal cell carcinoma. *Cancer* 1986;57:1871-1879.
103. Dunnill M, Millard P, Oliver D: Acquired cystic disease of the kidneys: A hazard of long-term intermittent maintenance haemodialysis. *J Clin Pathol* 1977;30:868-877.
104. Hughson M, Hennigar G, McManus J: Atypical cysts, acquired renal cystic disease, and renal cell tumors in end stage dialysis kidneys. *Lab Invest* 1980;42:475-480.
105. Williams JC, Merguerian PA, Schned AR, Morrison PM: Acquired renal cystic disease and renal cell carcinoma in an allograft kidney. *J Urol* 1995;153:395-396.
106. Gregoire J, Torres V, Holley K, Farrow G: Renal epithelial hyperplastic and neoplastic proliferation in autosomal dominant polycystic kidney disease. *Am J Kid Dis* 1987;9:27-38.
107. Jemal A, Siegel R, Ward E, et al.: Cancer statistics, 2007. *CA Cancer J Clin* 2007;57(1):43-66.
108. Cajulis RS, Katz RL, Dekmezian R, et al.: Fine needle aspiration biopsy of renal cell carcinoma. *Acta Cytol* 1993;37:367-372.
109. Nurmi M, Tyrkko J, Puntala P, et al.: Reliability of aspiration biopsy cytology in the grading of renal adenocarcinoma. *Scand J Urol Nephrol* 1984;18:151-156.
110. Foster K, Prowse A, van den Berg A, et al.: Somatic mutations of the von Hippel-Lindau disease tumour suppressor gene in non-familial clear cell renal cell carcinoma. *Hum Mol Genet* 1994;3:2069-2173.
111. Gnarr JR, Tory K, Weng Y, et al.: Mutations of the VHL tumour suppressor gene in renal carcinoma. *Nature Genetics* 1994;7:85-90.
112. Shuin T, Kondo K, Torigoe S, et al.: Frequent somatic mutations and loss of heterozygosity of the von Hippel-Lindau tumor suppressor gene in primary human renal cell carcinoma. *Cancer Res* 1994;54:2852-2855.
113. Whaley JM, Naglich J, Gelbert L, et al.: Germ-line mutations in the von Hippel-Lindau tumor suppressor gene are similar to somatic von Hippel-Lindau aberrations in sporadic renal cell carcinoma. *Am J Hum Genet* 1994;55:1092-1102.
114. Hidvegi D, DeMay RM, Nunez-Alonso C, Nieman H: Percutaneous transperitoneal aspiration of renal adenocarcinoma guided by ultrasound. *Acta Cytol* 1979;23:467-470.
115. Weir M, Pitman MB: The vascular architecture of renal cell carcinoma in fine-needle aspiration biopsies. An aid in its distinction from hepatocellular carcinoma. *Cancer* 1997;81(1):45-50.
116. Mancilla-Jimenez R, Stanley R, Blath R: Papillary renal cell carcinoma. A clinical, radiologic, and pathologic study of 34 cases. *Cancer* 1976;38:2469-2480.
117. Mydlo J, Bard R: Analysis of papillary renal adenocarcinoma. *Urology* 1987;30:529-534.
118. Kovacs G: Molecular differential pathology of renal cell tumours. *Histopathology* 1993;22:1-8.
119. Lager DJ, Huston BJ, Timmerman TG, Bonsib SM: Papillary renal tumors. *Cancer* 1995;76:669-673.
120. Kovacs G, Kovacs A: Parenchymal abnormalities associated with papillary renal cell tumors: A morphologic study. *J Urol Pathol* 1993;1:301-312.
121. Amin MB, Corless CL, Renshaw AA, et al.: Papillary (chromophil) renal cell carcinoma: Histomorphologic characteristics and evaluation of conventional pathologic prognostic parameters in 62 cases. *Am J Surg Pathol* 1997;21:621-635.
122. Gatalica Z, Kovatich A, Miettinen M: Consistent expression of cytokeratin 7 in papillary renal cell carcinoma. *J Urol Pathol* 1995;3:205-211.
123. Dekmezian R, Sneige N, Shabb N: Papillary renal-cell carcinoma: Fine-needle aspiration of 15 cases. *Diagn Cytopathol* 1991;7:198-203.

124. Flint A, Cookingham C: Cytologic diagnosis of the papillary variant of renal-cell carcinoma. *Acta Cytol* 1987;31:325-329.
125. Wang S, Filipowicz EA, Schnadig VJ: Abundant intracytoplasmic hemosiderin in both histiocytes and neoplastic cells: A diagnostic pitfall in fine-needle aspiration of cystic papillary renal cell carcinoma. *Diagn Cytopathol* 2001;24:82-85.
126. Weaver MG, Al-Kaisi N, Abdul-Karim FW: Fine needle aspiration cytology of a renal cell adenocarcinoma with massive intracellular hemosiderin accumulation. *Diagn Cytopathol* 1991;7:147-149.
127. Bonsib S, Lager D: Chromophobe cell carcinoma: analysis of five cases. *Am J Surg Pathol* 1990;14:260-267.
128. Thoenes W, Storkel S, Rumpelt H: Human chromophobe cell renal carcinoma. *Virchows Archiv B Cell Pathol* 1985;48:207-217.
129. Thoenes W, Storkel S, Rumpelt H, et al.: Chromophobe cell renal carcinoma and its variants—A report on 32 cases. *J Pathol* 1988;155:277-287.
130. Kovacs A, Kovacs G: Low chromosome number in chromophobe renal cell carcinoma. *Genes Chromosomes Cancer* 1992;4:267-268.
131. Kovacs G, Soudah B, Hoene E: Binucleated cells in a human renal cell carcinoma with 34 chromosomes. *Cancer Genet Cytogenet* 1988;31:211-215.
132. Speicher M, Schoell B, du Manoir S, et al.: Specific loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 in chromophobe renal cell carcinomas revealed by comparative genomic hybridization. *Am J Pathol* 1994;145:356-364.
133. Akhtar M, Kardar H, Linjawi T, et al.: Chromophobe cell carcinoma of the kidney. A clinicopathologic study of 21 cases. *Am J Surg Pathol* 1995;19:1245-1256.
134. Crotty TB, Farrow GM, Lieber MM: Chromophobe cell renal carcinoma: Clinicopathologic features of 50 cases. *J Urol* 1995;154:964-967.
135. Renshaw AA, Henske EP, Loughlin KR, et al.: Aggressive variants of chromophobe renal cell carcinoma. *Cancer* 1996;78:1756-1761.
136. Akhtar M, Ali MA: Aspiration cytology of chromophobe cell carcinoma of the kidney. *Diagn Cytopathol* 1995;13:287-294.
137. Renshaw AA, Granter SR: Fine needle aspiration of chromophobe renal cell carcinoma. *Acta Cytol* 1996;40:867-872.
138. Wiatrowska BA, Zakowski MF: Fine-needle aspiration biopsy of chromophobe renal cell carcinoma and oncocytoma. *Cancer Cytopathol* 1999;87:161-167.
139. Salamanca J, Alberti N, Lopez-Rios F, et al.: Fine needle aspiration of chromophobe renal cell carcinoma. *Acta Cytol* 2007;51(1):9-15.
140. Bonsib SM, Fischer J, Plattner S, Fallon B: Sarcomatoid renal tumors. *Cancer* 1987;59:527-532.
141. Ro JY, Ayala AG, Sella A, et al.: Sarcomatoid renal cell carcinoma: Clinicopathologic. A study of 42 cases. *Cancer* 1987;59:516-526.
142. Auger M, Katz RL, Sella A, et al.: Fine-needle aspiration cytology of sarcomatoid renal cell carcinoma: A morphologic and immunocytochemical study of 15 cases. *Diagn Cytopathol* 1993;9:46-51.
143. Baer SC, Ro JY, Ordonez NG, et al.: Sarcomatoid collecting duct carcinoma: A clinicopathologic and immunohistochemical study of five cases. *Hum Pathol* 1993;24:1017-1022.
144. Becht E, Muller SC, Storkel S, Alken P: Distal nephron carcinoma: A rare kidney tumor. *Eur Urol* 1988;14:253-254.
145. Carter MD, Tha S, McLoughlin MG, Owen DA: Collecting duct carcinoma of the kidney: A case report and review of the literature. *J Urol* 1992;147:1096-1098.
146. Cromie LJ, Davis CJ, DeTure FA: Atypical carcinoma of kidney. *Urology* 1979;13:315-317.
147. Fleming S, Lewi H: Collecting duct carcinoma of the kidney. *Histopathology* 1986;10:1131-1141.
148. Fuzesi L, Cober M, Mittermayer C: Collecting duct carcinoma: cytogenetic characterization. *Histopathology* 1992;21:155-160.
149. Kennedy S, Merino M, Linehan W, et al.: Collecting duct carcinoma of the kidney. *Hum Pathol* 1990;21:449-456.
150. Kotkawa Y, Sakamoto N, Saito S, et al.: Bellini duct carcinoma of the kidney. *Eur Urol* 1992;22:171-173.
151. Mauri MF, Bonzanini M, Luciani L, Palma PD: Renal collecting duct carcinoma. Report of a case with urinary cytologic findings. *Acta Cytol* 1994;38:755-758.
152. Rumpelt HJ, Storkel S, Moll R, et al.: Bellini duct carcinoma: Further evidence for this rare variant of renal cell carcinoma. *Histopathology* 1991;18:115-122.
153. Zaman SS, Sack MJ, Ramchandani P, et al.: Cytopathology of retrograde renal pelvis brush specimens: collecting duct carcinoma and low-intermediate grade transitional cell carcinoma. *Diagn Cytopathol* 1996;15:312-321.
154. Davis CJ, Mostofi FK, Sesterhenn IA: Renal medullary carcinoma. The seventh sickle cell nephropathy. *Am J Surg Pathol* 1995;19:1-11.
155. Gregori-Romero MA, Morell-Quadreny L, Llombart-Bosch A: Cytogenetic analysis of three primary Bellini duct carcinomas. *Genes, Chromosomes & Cancer* 1996;15:170-172.
156. Renshaw AA, Maurici D, Fletcher JA: Papillary renal cell carcinoma with rare papillae histologically resembling collecting duct carcinoma. *J Urol Pathol* 1996;5:65-74.
157. Bejar J, Szvalb S, Maly B, et al.: Collecting duct carcinoma of the kidney: A cytological study and case report. *Diagn Cytopathol* 1996;15:136-138.
158. Caraway NP, Wojcik EM, Katz RL, et al.: Cytologic findings of collecting duct carcinoma of the kidney. *Diagn Cytopathol* 1995;13:304-309.
159. Layfield LJ: Fine-needle aspiration biopsy of renal collecting duct carcinoma. *Diagn Cytopathol* 1994;11:74-78.
160. Sarode VR, Islam S, Wooten D, et al.: Fine needle aspiration cytology of collecting duct carcinoma of the kidney: Report of a case with distinctive features and differential diagnosis. *Acta Cytol* 2004;48:843-848.
161. Sironi M, Delpiano C, Claren R, Spinelli M: New cytological findings on fine-needle aspiration of renal collecting duct carcinoma. *Diagn Cytopathol* 2003;29:239-240.
162. Renshaw AA, Granter SR, Fletcher JA, et al.: Renal cell carcinomas in children and young adults. *Am J Surg Pathol* 1999;23:795-802.
163. Argani P, Antonescu CR, Fournet JC, et al.: Renal cell carcinomas with the t(X;1)(p11.2;q21): Detailed morphologic, immunohistochemical, ultrastructural and molecular analysis of a cytogenetically-defined entity (abstract). *Mod Pathol* 2002;15:153A.
164. Argani P, Antonescu CR, Illei PB, et al.: Primary renal neoplasms with the ASPL-TFE3 gene fusion of alveolar soft part sarcoma: A distinctive tumor entity previously included among renal cell carcinomas of children and adolescents. *Am J Pathol* 2001;159:179-192.
165. Gupta K, Nijhawan R: Renal-cell carcinoma in a three-year-old child (letter). *Diagn Cytopathol* 2004;30:369-370.
166. Schinstine M, Filie AC, Torres-Cabala C, et al.: Fine-needle aspiration of a Xp11.2 translocation/TFE3 fusion renal cell carcinoma metastatic to the lung: report of a case and review of the literature. *Diagn Cytopathol* 2006;34:751-756.
167. Santamaria M, Jauregui I, Urtasun F, Bertol A: Fine needle aspiration biopsy in urothelial carcinoma of the renal pelvis. *Acta Cytol* 1995;39:443-448.

168. Friedman HD, Nsoulis IS, Krauss DJ, et al.: Transitional cell carcinoma arising in a pyelocaliceal cyst. An unusual cystic renal lesion with cytologic and imaging findings. *Virchows Arch* 1999;434:459-462.
169. Powers CN, Elbadawi A: "Cercariform" cells: A clue to the cytodiagnosis of transitional cell origin in metastatic neoplasms? *Diagn Cytopathol* 1995;13:15-21.
170. Renshaw AA, Madge R: Cercariform cells for helping distinguish transitional cell carcinoma from non-small cell lung carcinoma in fine needle aspirates. *Acta Cytol* 1997;41:999-1007.
171. Bracken RB, Chica G, Johnson DE: Secondary renal neoplasms, an autopsy study. *South Med J* 1979;72:806-807.
172. Gattuso P, Ramzy I, Truong LD, et al.: Utilization of fine-needle aspiration in the diagnosis of metastatic tumors to the kidney. *Diagn Cytopathol* 1999;21:35-38.
173. Giashuddin S, Cangiarrella J, Elgert P, Levine PH: Metastases to the kidney: Eleven cases diagnosed by aspiration biopsy with histologic correlation. *Diagn Cytopathol* 2005;32:325-329.
174. Khurana KK, Powers CN: Basaloid squamous carcinoma metastatic to renal-cell carcinoma: Fine needle aspiration cytology of tumor-to-tumor metastasis. *Diagn Cytopathol* 1997;17:379-382.
175. Tabatabai ZL, Staerckel GA: Distinguishing primary and metastatic conventional renal cell carcinoma from other malignant neoplasms in fine-needle aspiration biopsy specimens. *Arch Pathol Lab Med* 2005;129:1017-1021.
176. Simsir A, Chhieng D, Wei XJ, et al.: Utility of CD10 and RCCm in the diagnosis of metastatic conventional renal-cell adenocarcinoma by fine-needle aspiration biopsy. *Diagn Cytopathol* 2005;33:3-7.
177. Grignon DJ, Ro JY, Ayala AG: Primary mucin-secreting adenocarcinoma of the kidney. *Arch Pathol Lab Med* 1988;112:847-849.
178. Capella C, Eusebi V, Rosai J: Primary oat cell carcinoma of the kidney. *Am J Surg Pathol* 1984;8:855-861.
179. Ferry JA, Harris NL, Papanicolaou N, Young RH: Lymphoma of the kidney. *Am J Surg Pathol* 1995;19:134-144.
180. Silverman SG, Mueller PR, Pinkney LP, et al.: Predictive value of image-guided adrenal biopsy: Analysis of results of 101 biopsies. *Radiology* 1993;187(3):715-718.
181. DeAgustin P, Lopez-Rios F, Alberti N, et al.: Fine needle aspiration biopsy of the adrenal glands: A ten year experience. *Diagn Cytopathol* 1999;21:92-97.
182. Dusenbery D, Dekker A: Needle biopsy of the adrenal gland: retrospective review of 54 cases. *Diagn Cytopathol* 1996;14:126-134.
183. Kindelberger D, Cibas ES: Evaluating metastatic carcinoma to the adrenal: Utility of fine needle aspiration. *Pathology Case Reviews* 2005;10:257-262.
184. Fassina AS, Borsato S, Fedeli U: Fine needle aspiration cytology (FNAC) of adrenal masses. *Cytopathol* 2000;11:302-311.
185. Deodhare S, Saap M: Adrenal histoplasmosis: Diagnosis by fine needle aspiration biopsy. *Diagn Cytopathol* 1997;17:42-44.
186. NIH State-of-the-Science statement on management of the clinically inapparent adrenal mass ("incidentaloma"). *NIH Consens State Sci Statements* 2002 Feb. 4-6;19(2):1-23.
187. McCorkell SJ, Niles NL: Fine-needle aspiration of catecholamine-producing adrenal masses: a possibly fatal mistake. *AJR Am J Roentgenol* 1985;145(1):113-114.
188. Min KW, Song J, Boesenberg M, et al.: Adrenal cortical nodule mimicking small round cell malignancy on fine needle aspiration. *Acta Cytol* 1988;32:543-546.
189. Suen KC, McNeely TB: Adrenal cortical cells mimicking small cell anaplastic carcinoma in a fine-needle aspirate. *Mod Pathol* 1991;4(5):594-595.
190. Saboorian MH, Katz RL, Charnsangavej C: Fine needle aspiration cytology of primary and metastatic lesions of the adrenal gland. A series of 188 biopsies with radiologic correlation. *Acta Cytol* 1995;39(5):843-851.
191. Settakorn J, Sirivanichai C, Rangdaeng S, et al.: Fine needle aspiration cytology of adrenal myelolipoma: Case report and review of the literature. *Diagn Cytopathol* 1999;21:409-412.
192. Medeiros LJ, Weiss LM: New developments in the pathologic diagnosis of adrenal cortical neoplasms. A review. *Am J Clin Pathol* 1992;97:73-83.
193. Weiss LM: Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. *Am J Surg Pathol* 1984;8:163-169.
194. Aubert S, Wacrenier A, Leroy X, et al.: Weiss system revisited: A clinicopathologic and immunohistochemical study of 49 adrenocortical tumors. *Am J Surg Pathol* 2002;26(12):1612-1619.
195. Wu HH, Cramer HM, Kho J, et al.: Fine needle aspiration cytology of benign adrenal cortical nodules. A comparison of cytologic findings with those of primary and metastatic adrenal malignancies. *Acta Cytol* 1998;42:1352-1358.
196. Chen KT: Extraneous cells of hepatic origin in adrenal fine needle aspiration as a diagnostic pitfall: a case report. *Acta Cytol* 2005;49(4):449-451.
197. Baguet JP, Hammer L, Tremel F, et al.: Metastatic pheochromocytoma: Risks of diagnostic needle puncture and treatment by arterial embolization. *J Hum Hypertens* 2001;15:209-211.
198. Jorda M, De MB, Madji M: Calretinin and inhibin are useful in separating adrenocortical neoplasms from pheochromocytomas. *Appl Immunohistochem Mol Morph* 2002;10:67-70.
199. Wadih GE, Nance KV, Silverman JF: Fine-needle aspiration cytology of the adrenal gland. Fifty biopsies in 48 patients. *Arch Pathol Lab Med* 1992;116(8):841-846.
200. Chu P, Wu E, Weiss LM: Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. *Mod Pathol* 2000;13:962-972.
201. Fetsch PA, Powers CN, Zakowski MF, et al.: Anti-alpha-inhibin: marker of choice for the consistent distinction between adrenocortical carcinoma and renal cell carcinoma in fine needle aspiration. *Cancer* 1999;87:168-172.
202. Loy TS, Phillips RW, Linder CL: A103 immunostaining in the diagnosis of adrenal cortical tumors: An immunohistochemical study of 316 cases. *Arch Pathol Lab Med* 2002;126:170-172.
203. Renshaw AA, Granter SR: Comparison of A103 and inhibin reactivity in adrenal cortical tumors: Distinction from hepatocellular carcinoma and renal tumors. *Mod Pathol* 1998;11:1160-1164.
204. Rishi M, Howard LN, Bratthauer GL, Tavassoli FA: Use of monoclonal antibody against human inhibin as a marker for sex cord stromal tumors of the ovary. *Am J Surg Pathol* 1997;21:583-589.
205. Yang B, Ali SZ, Rosenthal DL: CD10 facilitates the diagnosis of metastatic renal cell carcinoma from primary adrenal cortical neoplasms in adrenal fine needle aspiration. *Diagn Cytopathol* 2002;27:149-152.
206. Lam KY, Lo CY: Metastatic tumours of the adrenal glands; a 30 year experience in a teaching hospital. *Clin Endocrinol* 2002;56:95-101.

Ovary

EDMUND S. CIBAS

OBTAINING THE SPECIMEN

PREPARING THE SPECIMEN AND REPORTING RESULTS

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Endometriotic Cyst

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UNCOMMON PRIMARY OVARIAN TUMORS

METASTATIC TUMORS

Fine-needle aspiration (FNA) is a safe, minimally invasive, and relatively inexpensive procedure for the diagnosis of selected ovarian lesions. Indeed, with the widespread use of ultrasound and laparoscopy, many more ovarian lesions are being detected and aspirated than ever before.

Indications for fine-needle aspiration of the ovary:

- confirm the benign nature of an incidental cyst discovered during:
 - an infertility work-up
 - pregnancy
- confirm malignancy in a patient with an inoperable pelvic mass
- confirm recurrence of an ovarian tumor treated conservatively
- drain a tubo-ovarian abscess

FNA of the ovary is generally reserved for small, incidental cystic masses that appear benign on sonographic

or laparoscopic examination.¹⁻³ Incidental ovarian cysts are often discovered in women with infertility or during pregnancy.⁴ Aspiration cytology, in combination with a benign ultrasound appearance, is used to reassure the patient that an oophorectomy is not necessary.⁵ In some laboratories, estradiol (E2) levels in the fluid are also measured because these are usually elevated in follicle-derived cysts but not in epithelial lesions.^{6,7} Thus, ultrasound, cytology, and E2 levels can form an effective “triple test” for distinguishing benign from malignant ovarian cysts.

Ultrasound features of a benign ovarian cyst:

- small
- thin walled
- no septations
- no papillarity
- no solid areas
- low echogenicity (sonolucency)

As a result of this preselection, the great majority of ovarian FNAs are benign.⁸ Nevertheless, neoplastic epithelial cysts are occasionally encountered, and the diagnosis of endometriosis is sometimes established by this means as an unsuspected cause of infertility.⁴

If any ultrasound features are worrisome, the physician will usually forego aspiration and recommend surgery. Aspiration of suspicious ovarian masses should be avoided, except to confirm malignancy in patients with inoperable or metastatic disease.^{3,9} There is a risk, albeit slight, of tumor seeding of the peritoneal cavity. Because malignant tumors are treated by surgical resection, FNA is redundant. Finally, because of the possibility of sampling error, a benign FNA result in the face of worrisome ultrasound features does not exclude malignancy.

OBTAINING THE SPECIMEN

The aspiration can be carried out transrectally, transvaginally, percutaneously, intraoperatively,¹⁰ or during laparoscopy. The method used depends on the size of the lesion, its location, and the resources available to the person performing the aspiration. Percutaneous transabdominal aspiration can be performed by palpation,¹¹ or more commonly, with imaging guidance. Transvaginal and transrectal aspiration can be done by palpation using the Franzen needle guide¹¹ originally developed for FNA of the prostate. Transvaginal aspiration is often performed using ultrasound guidance with the probe placed in the vagina and can be done in the outpatient setting with only intravenous sedation.^{4,8} Depending on the trajectory of the needle, the cyst fluid may be contaminated with squamous cells, mesothelial cells, urothelial cells, or intestinal epithelium. Therefore, knowledge of the route taken is important in evaluating the specimen.^{8,12-14} Aspirations are also performed when cysts are discovered during laparoscopy and laparotomy.

Complications are uncommon. Although common wisdom holds that puncture of a malignant tumor can cause seeding of the peritoneal cavity, documented cases are actually rare.¹⁵ Some authors with a large experience have reported no such cases.¹⁶ Severe pelvic infection after transvaginal and transrectal FNA is seen in 1.3% of cases, however.¹⁶ Acute abdominal or pelvic pain is a contraindication to FNA because it may delay treatment of a serious condition such as torsion of a cyst.¹

PREPARING THE SPECIMEN AND REPORTING RESULTS

Specimens are usually cyst fluids. Standard methods are used in preparation: sedimentation for direct smears; membrane filtration, cytocentrifugation; thin-

layer preparation; or cell block sectioning. A portion of the fresh fluid can be used to measure the level of E2 or tumor-associated antigens cancer antigen 125 (CA-125), carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP). Elevated levels are typical of some ovarian lesions and can be a useful adjunct to cytologic examination.^{16,17}

Specimens that are virtually acellular are best termed *non-diagnostic*.^{18,19} The percentage of FNAs of the ovary that are non-diagnostic ranges widely, from a low of 13% to a high of 72%,^{1,4,12,13,18-20} this variation is likely related to the type of lesion selected for aspiration and the definition of a nondiagnostic specimen. The cytology report should state that malignant cells are either absent (no malignant cells identified) or present (positive for malignant cells). If the findings are equivocal, the report should state either that atypical or suspicious cells are present. If sufficient benign lesional cells (granulosa cells, epithelial cells) are seen, descriptive terms can further categorize the cyst as a follicular, simple, serous, benign mucinous, endometriotic, or dermoid cyst.³ Benign ovarian FNA results fall into two broad categories: (1) follicular or lutein cysts, and (2) epithelial cysts. This has significant clinical relevance because surgery is unnecessary for follicle-derived cysts, which usually regress over time. Surgery may be indicated, however, for a benign-appearing epithelial cyst, particularly if the sonographic findings are worrisome. The lining of an epithelial cyst can vary from one area to another, and some cysts contain benign, borderline, and frankly malignant foci; sampling is not always representative of this heterogeneity. For this reason, an explanatory note accompanying the diagnosis of a benign epithelial cyst is helpful (e.g. "Clinical correlation is advised to ensure that the sample is representative of the underlying lesion").²¹

ACCURACY

Sensitivity for malignancy ranges from 84% to 93%, but these numbers are inflated because borderline tumors were excluded from analysis.^{12,16} In fact, FNA of a borderline tumor often yields a false-negative result^{4,16,22,23}; much of the lesion is acellular cyst fluid, and neoplastic epithelium may not be sampled. When borderline tumors have been included in the statistical analysis, the sensitivity is low, ranging from 26% to 40%.²²⁻²⁴ Low sensitivity is one of the strongest arguments against the aspiration of clinically suspicious ovarian masses.

False-positive results have been reported.^{12,24} A notorious cause of false-positives is the follicle cyst, which can yield a cellular specimen with mitotic activity.^{9,25,26} As mentioned previously, familiarity with the laparoscopic and sonographic findings can be critical in the

distinction between benign and malignant ovarian lesions by FNA.

FNA of the ovary, therefore, has its limitations. Not only is there a high false-negative rate for malignancy, particularly for borderline tumors, but the method cannot reliably distinguish between borderline tumors and carcinomas.^{18,27,28} (Because both lesions are treated by surgical resection, the inadequacy of FNA in this instance is not significant.) Furthermore, benign lesions cannot always be histologically categorized by cytologic evaluation.^{17,27} Although in some cases FNA can establish a specific diagnosis such as endometriosis,⁸ at present it is most valuable for confirming a clinical impression that an ovarian cyst is benign, thus sparing the patient unnecessary surgery.^{1,18,29}

BENIGN TUMOR-LIKE LESIONS OF THE OVARY

Non-Neoplastic Cysts

Benign ovarian cysts are common; the majority are discovered incidentally by ultrasonography, laparoscopy, or laparotomy. They fall into two major categories: the most common are the so-called functional cysts (i.e., cysts of follicular origin—follicle and corpus luteum cysts). The rest are nonfunctional cysts derived from ovarian surface epithelium or endometriosis. Precise classification by cytologic examination is not always possible, particularly when only cyst contents (fluid and macrophages) are obtained.³⁰

Cystic Follicle and Follicle Cyst

These two lesions have a similar histogenesis and are distinguished only on the basis of size. (The arbitrary size cutoff is variably given as 2 cm, 2.5 cm, and 3 cm; if the size cutoff is exceeded, a cystic follicle becomes a follicle cyst.) One of the most common, if not the most common, cystic lesions of the ovary, they arise from an ovarian follicle and are not neoplastic but rather physiologic. Ovarian follicles follow a predictable development from primordial follicle (oocyte surrounded by a flattened layer of granulosa cells) to primary follicle (increased size and mitotic activity of granulosa cells) to secondary follicle (stratification of granulosa cells) to the Graafian follicle (characterized by a distinct zone of fluid, an antrum for the oocyte, and proliferation of surrounding theca cells). Cystic follicles and follicle cysts occur when a Graafian follicle does not rupture but rather persists for a variable period of time. The remainder of this discussion focuses on the follicle cyst, which, because of its size, is more likely to manifest clinically.

Follicle cysts can be solitary or multiple and range in size up to 8 cm or more in diameter. They look benign on sonographic and laparoscopic examination; they are unilocular, smooth-surfaced, translucent, and thin-walled. Most regress spontaneously within a few months. Multiple follicle cysts are common in juvenile hypothyroidism, ovarian hyperstimulation syndrome, and polycystic ovary disease.

Histologically, a follicle cyst is lined by an inner layer of attenuated or stratified granulosa cells and an outer layer of theca cells (Fig. 15.1); both may show

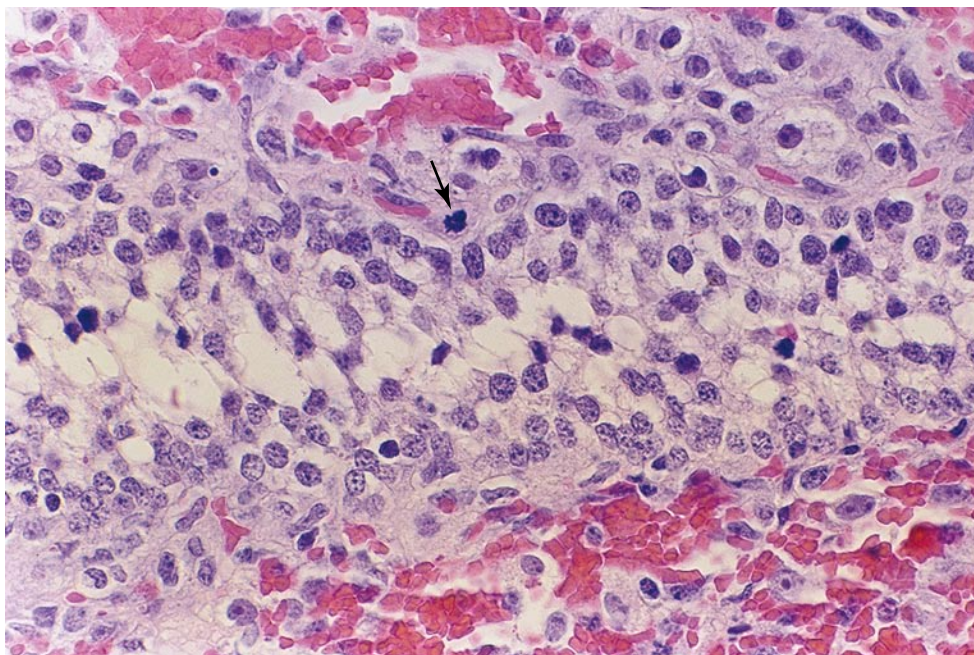


Figure 15.1 Follicle cyst. In this histologic section, the follicle cyst is collapsed and the cavity slitlike. Follicle cysts are lined by one or several layers of granulosa cells. Mitotic activity (arrow) is common (hematoxylin-eosin [H & E] stain).

luteinization. Fluid is clear or hemorrhagic. It may be sparsely cellular, containing only histiocytes and blood, or markedly cellular (sometimes alarmingly so), with numerous granulosa cells that are isolated and in large clusters.

Because follicle cysts appear benign sonographically and laparoscopically, they are managed conservatively, with an eye on preserving the ovary. FNA is commonly used as a minimally invasive method for sampling the lesion and confirming that it is in fact benign.

CYTOMORPHOLOGY OF FOLLICLE CYST:

- sparsely or highly cellular
- isolated cells and large clusters
- coarsely granular chromatin
- mix of viable and pyknotic nuclei
- foamy cytoplasm
- mitoses

FNA specimens range from sparsely to highly cellular. The highly cellular cases are termed *cellular follicle cysts* and account for between 23% and 76% of all follicle cysts.^{18,31} The granulosa cells can be isolated, but they have a tendency to cluster haphazardly in loose spherical aggregates (Fig. 15.2). They have a round nucleus with coarsely granular chromatin and one or two small nucleoli. Nuclear grooves are not usually seen.²⁵ Some nuclei are dark and pyknotic and eccentrically placed, whereas others are round and vesicular with a prominent nucleolus. Mitotic figures can be absent or numerous (Fig. 15.3). Luteinized granulosa cells have foamy cytoplasm that may contain yellow pigment. Theca interna cells have an eccentrically placed ovoid nucleus and a moderate amount of cytoplasm. Theca externa cells are indistinguishable from spindle-shaped ovarian stromal cells.³²

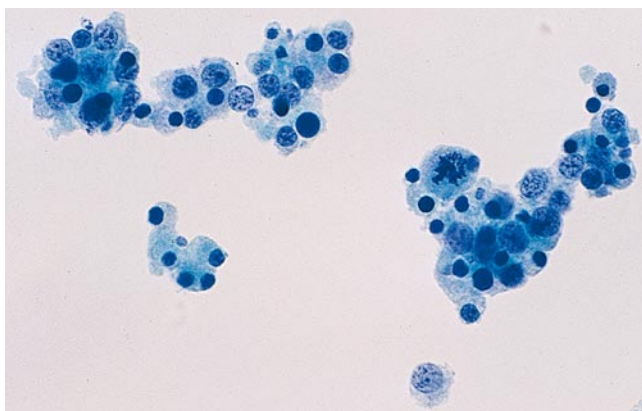


Figure 15.2 Follicle cyst. Granulosa cells are clustered in loose aggregates (Papanicolaou stain).

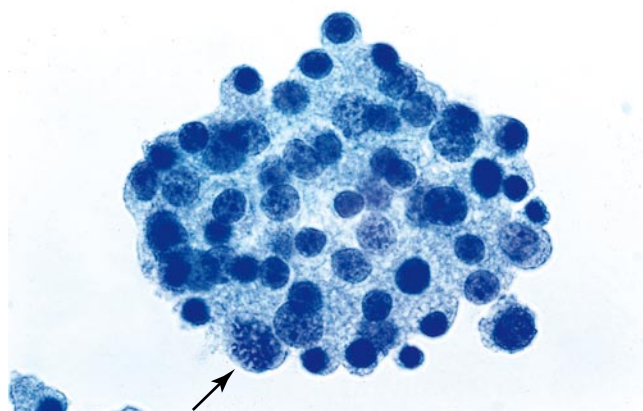


Figure 15.3 Follicle cyst. Granulosa cells have round nuclei and a moderate amount of granular cytoplasm. Degenerated granulosa cells with pyknotic nuclei are a common finding, as are mitoses (arrow; Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF FOLLICLE CYST:

- epithelial cysts
 - simple cyst
 - serous cyst
 - mucinous cyst
 - endometriotic cyst
- granulosa cell tumor
- carcinoma

Sparsely cellular follicle cysts are indistinguishable from benign, nonfollicular cysts such as simple and serous ovarian cysts. When ciliated or mucinous epithelium is identified, the cyst is of surface epithelial origin and not a follicle cyst. E2 levels in cyst fluid as measured by radioimmunoassay are helpful; elevated E2 concentration correlates strongly with cysts of follicular origin.^{1,6,7,16} Eighty-one percent to 90% of follicle cysts have an E2 content greater than 20 nmol/L, and in 97% to 99% of nonfollicular cysts the E2 content is less than 20 nmol/L. Also helpful are fluid CEA and CA-125 levels, which are low in follicle cysts; one or more of these is usually elevated in epithelial cysts such as serous, mucinous, and endometriotic cysts.^{17,29} The differential diagnosis also includes a cystic granulosa cell tumor. Fluid from the latter is usually highly cellular.³³ Unlike the cells of a follicle cyst, those of a granulosa cell tumor have nuclei with pale, finely dispersed chromatin. Cellular follicle cysts are a potential pitfall: Because of their cellularity and conspicuous mitotic activity, they may raise the possibility of a granulosa cell tumor or carcinoma.^{9,25,26} Knowledge of the invariably benign sonographic and laparoscopic findings of follicle cysts, including the cellular variant, is reassuring and diagnostically helpful.

Corpus Luteum Cyst

Corpus luteum cysts are unilocular and histologically similar to follicle cysts except that the wall is composed of large luteinized granulosa and theca interna cells.

CYTOMORPHOLOGY OF CORPUS LUTEUM CYST:

- isolated large cells
- abundant foamy cytoplasm
- intact or pyknotic nuclei
- macrophages
- hyaline bodies, calcification (pregnancy)

The fluid may be hemorrhagic and composed of numerous, predominantly isolated luteinized granulosa cells with round or pyknotic nuclei. The cytoplasm is abundant, finely vacuolated, and contains lipid (Fig. 15.4). Macrophages with hemosiderin or the

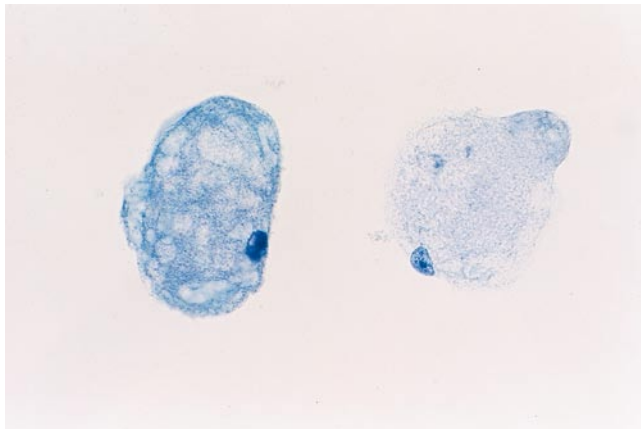


Figure 15.4 Corpus luteum cyst. These cysts are lined by large cells that have a small nucleus and abundant finely vacuolated cytoplasm (Papanicolaou stain).

yellow hematoidin pigment and smaller luteinized theca interna cells are also seen.³⁴ Cysts associated with pregnancy contain hyaline bodies and calcifications.¹⁸ Fluid from a hemorrhagic corpus luteum cannot always be distinguished cytologically from that of other benign cysts, including endometriotic cysts.

Endometriotic Cyst

Endometriosis affects women of reproductive age and is often associated with infertility. It can result in a cystic tumor-like mass of the ovary, known as an endometriotic cyst or endometrioma. As many as one half of ovarian endometriomas are bilateral. For an unequivocal diagnosis, identification of two of the following features is required: endometrial glands, endometrial stroma, and hemosiderin.

CYTOMORPHOLOGY OF ENDOMETRIOTIC CYST:

- hemosiderin-laden macrophages
- endometrial glandular cells
- endometrial stromal cells

The aspirated cyst fluid contains hemolyzed old blood the color of chocolate (“chocolate cyst”). Hemosiderin-laden macrophages are numerous (Fig. 15.5A). Endometrial epithelial cells are usually arranged in clusters or sheets. The cells are small and have indistinct borders; nuclei are round or oval; nucleoli are inconspicuous; and cytoplasm is scant and sometimes vacuolated. Nuclear molding may be seen. Stromal cells with oval nuclei and scant cytoplasm may also be present. Epithelial and stromal cells are best appreciated as such on cell block preparations (Fig. 15.5B).

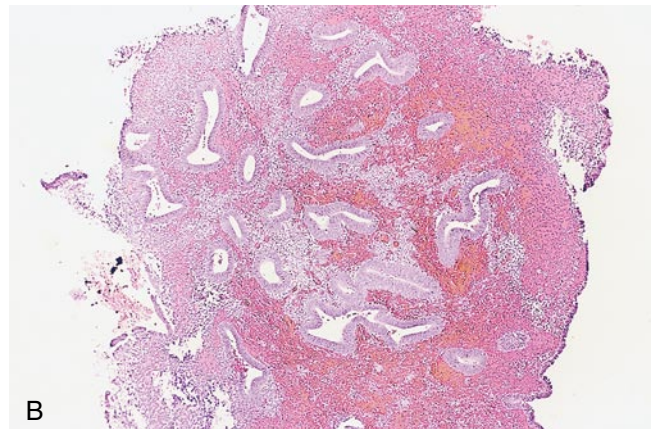
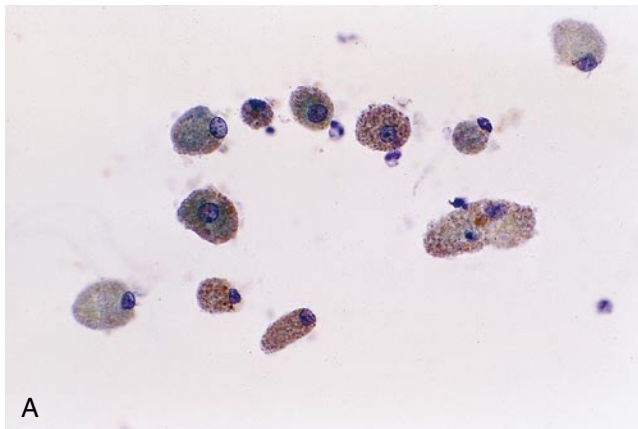


Figure 15.5 Endometriotic cyst. A, Cytologic preparations often show only macrophages with abundant intracytoplasmic hemosiderin (Papanicolaou stain). B, In cell block sections, fragments containing endometrial glands and stroma are diagnostic (hematoxylin-eosin [H & E] stain).

The diagnosis of endometriosis is easily made when both cytologically benign epithelial and stromal cells are present. It is impossible to distinguish an endometriotic cyst from a hemorrhagic corpus luteum when epithelial cells are absent because luteinized granulosa and theca cells resemble histiocytes.³⁵ Occasional endometriotic cysts have marked cytologic atypia, and flow cytometric DNA measurements have demonstrated aneuploidy in 50% of such cases,³⁶ causing some investigators to speculate that they may be precursors to ovarian carcinoma. A suspicion of malignancy may be unavoidable in these atypical endometriotic cysts.

Simple Ovarian, Parovarian, and Paratubal Cysts

Simple cysts of the ovary or fallopian tube are most common in postmenopausal women and result from an invagination of mesothelium or the surface epithelium. They are usually small and multiple and lined by a single layer of benign columnar, cuboidal, or flattened epithelium. When the epithelium is ciliated, the cyst is called a serous cyst.

CYTOMORPHOLOGY OF SIMPLE CYSTS:

- scant cellularity
- mesothelial-like cells
- sheets and clusters

The cytologic features of simple ovarian, parovarian, and paratubal cysts are identical. They contain clear fluid that is sparsely cellular. When small cuboidal epithelial cells are seen (rarely), they are usually arranged in tight clusters or sheets. The cells resemble benign mesothelial cells (Fig. 15.6), having a small round or oval nucleus,

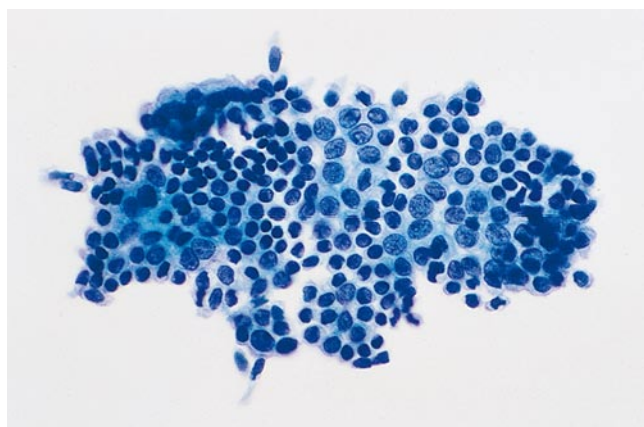


Figure 15.6 Simple ovarian cyst. This sheet of evenly arranged cuboidal cells resembles a sheet of mesothelial cells (Papanicolaou stain).

finely granular chromatin and an inconspicuous nucleolus. The quantity of cytoplasm varies. If ciliated cells are present, however, the lesion is either a serous cyst or a hydrosalpinx.

Hydrosalpinx

Hydrosalpinx, a complication of salpingitis, presents as a large cystic adnexal mass. The distended fallopian tube is filled with clear fluid and is lined by ciliated epithelium. The fluid is hypocellular and may contain histiocytes, ciliated epithelial cells, or detached ciliary tufts (Fig. 15.7). These findings are identical to those seen with serous ovarian and parovarian cysts.

Tubo-Ovarian Abscess

Tubo-ovarian abscess is an advanced complication of acute salpingitis, known clinically as pelvic inflammatory disease. It presents as a palpable adnexal mass that can be visualized by ultrasonography or computed tomography (CT). Most cases result from an ascending infection of the lower genital tract by sexually transmitted pathogens, of which the most common are *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Mycoplasma genitalium*.

Aspirated material is thick and yellow and composed of numerous polymorphonuclear leukocytes and abundant necrotic debris. When these elements are seen during a rapid interpretation of the aspirated material, additional passes should be performed to obtain samples for microbiologic study to identify the causative organism. Surgery is considered for those patients who do not respond to antibiotic therapy. Laparoscopic or radiographically guided drainage is helpful in some cases.

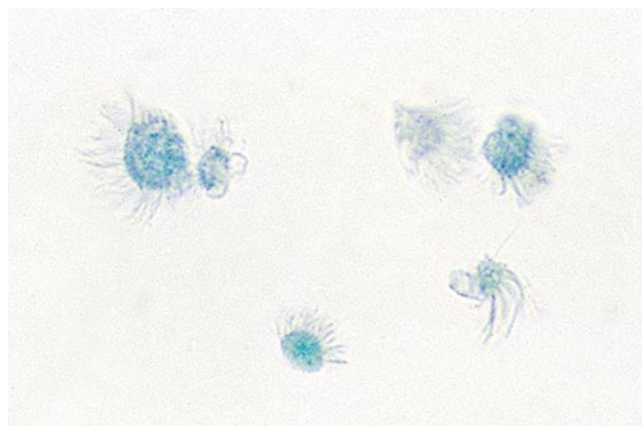


Figure 15.7 Detached ciliary tufts. These decapitated tips of ciliated cells are diagnostically useful because they exclude a follicle cyst. They are seen in serous cysts, cystic teratomas, and hydrosalpinx (Papanicolaou stain).

BENIGN SURFACE EPITHELIAL-STROMAL TUMORS

Tumors derived from the ovarian surface epithelium are the most common neoplasms of the ovary. They are divided histologically into serous (the most common), mucinous, endometrioid, clear cell, transitional, squamous, mixed, and undifferentiated types. Most of these have a benign, borderline, and malignant counterpart. Because FNA is rarely used to diagnose these neoplasms, only the more common types are discussed next.

Benign Serous Tumors

Benign serous tumors (e.g., serous cystadenoma, serous adenofibroma, and serous cystadenofibroma) constitute about 16% of benign ovarian neoplasms.³⁷ They are defined as tumors composed of epithelium resembling either the fallopian tube lining or ovarian surface. They are usually cystic, either unilocular or multilocular, and the cysts contain clear fluid. The cyst wall may be entirely smooth and round or nodular. They are lined by ciliated or sometimes nonciliated columnar cells. Some benign serous tumors have an additional stromal component, hence adenofibroma and cystadenofibroma. The majority of ovarian adenofibromas are serous, but endometrioid, mucinous, and clear cell differentiation are occasionally seen.²¹

CYTOMORPHOLOGY OF A BENIGN SEROUS TUMOR:

- ciliated cells
- cuboidal cells
- detached ciliary tufts
- psammoma bodies (rare)

FNA of a benign cystic serous tumor yields clear fluid that is usually sparsely cellular but occasionally highly cellular. The cuboidal cyst lining cells, if present, have a uniformly round or oval nucleus and are arranged in crowded clusters. Ciliated columnar cells (Fig. 15.8), psammoma bodies, and detached ciliary tufts (see Fig. 15.7) are sometimes present.³⁸ If the needle has collected a sample from a solid area of an adenofibroma, stromal cells may be seen.

If the cyst fluid does not contain ciliated cells or detached ciliary tufts, the findings are non-specific. Analysis of tumor markers may be helpful because elevated CA-125 levels are characteristic of serous cysts.^{1,16,17,29}

Benign Mucinous Tumors

Twenty percent of benign ovarian neoplasms are mucinous tumors (i.e., mucinous cystadenoma, cystadenofibroma, and adenofibroma). Most are seen in women of reproductive age. The most common subtype by far is the mucinous cystadenoma, which is usually large and multiloculated. The wall is smooth and lined by a single layer of endocervical-like columnar cells or intestinal-type cells, predominantly goblet cells.

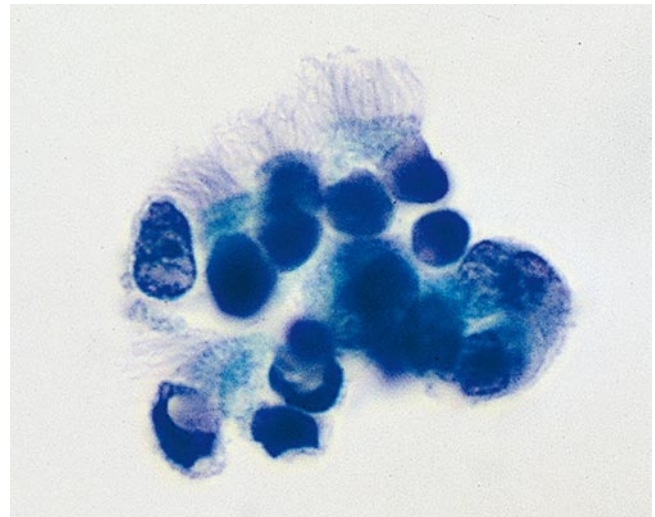


Figure 15.8 Serous cystadenoma. The cyst is lined by benign ciliated cells that have basally placed nuclei, terminal bars, and cilia (Papanicolaou stain).

CYTOMORPHOLOGY OF MUCINOUS CYSTADENOMA:

- mucinous cells
 - endocervical-like cells
 - goblet cells
- isolated cells, ribbons, sheets
- macrophages
- extracellular mucin

The fluid is either gelatinous or watery. The lining cells are isolated, often retaining their columnar shape, or arranged in clusters and honeycomb-like sheets with sharply defined cell membranes. They resemble either benign endocervical cells (Fig. 15.9) or goblet cells (Fig. 15.10). Mucin-filled macrophages and extracellular mucin are present.

Diagnosing a mucinous cystadenoma is straightforward when mucinous epithelium is identified. If absent, the findings are non-specific. If needed, chemical analysis of the fluid can be helpful: A high CEA level and low E2 and CA-125 levels are characteristic of mucinous cysts and help to distinguish them from follicle and serous cysts.^{1,16,17,29} The differential diagnosis also includes a mucinous carcinoma and a mucinous borderline tumor. If nuclear atypia is present, a borderline tumor or carcinoma should be suspected. Surgical excision of the cyst

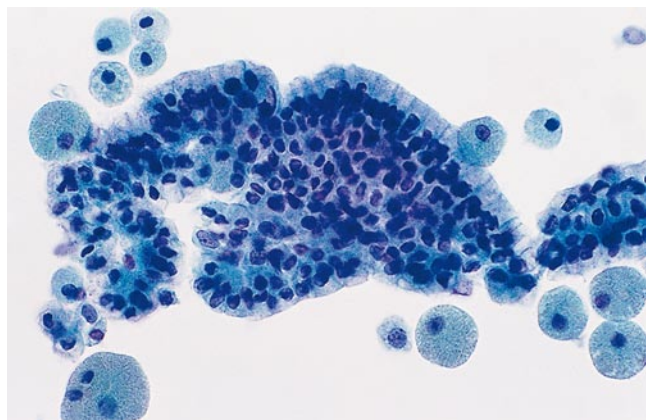


Figure 15.9 Mucinous cystadenoma. Admixed with macrophages are fragments of benign mucinous epithelium resembling endocervical epithelium (Papanicolaou stain).

should be considered even when the cytologic findings are benign, because the borderline or malignant areas can be focal and may not be sampled by FNA.^{16,22,23}

Benign Brenner Tumor

Brenner tumors are a subtype of the transitional cell neoplasms of the ovary and account for about 2% of ovarian neoplasms. Most are benign, and less than 10% of cases occur bilaterally. Most Brenner tumors are solid, but some contain small or large cystic areas that may show mucinous differentiation.

Benign Brenner tumors are composed of nests of transitional epithelium embedded in a dense fibrous stroma.

Aspirates show sheets of transitional cells, the nuclei of which resemble coffee beans because they are oval and have a prominent longitudinal groove. Globular hyaline structures that stain bright orange with the Papanicolaou stain are seen³⁹; their composition and significance are not known. The differential diagnosis includes a granulosa cell tumor, the cells of which also have longitudinal grooves. Granulosa cell tumors, unlike Brenner tumors, are negative for keratin proteins.

MALIGNANT SURFACE EPITHELIAL-STROMAL TUMORS

FNA is rarely used to diagnose borderline and malignant ovarian tumors, for the reasons given previously: Most of these tumors already have a suspicious laparoscopic or imaging appearance that merits surgery; FNA has a significant false-negative rate for these tumors; and there is concern over cyst rupture with seeding of the peritoneum. Occasionally, however, some ovarian tumors present as pelvic masses so large they significantly alter pelvic anatomy. In such cases, an FNA might be performed because a soft tissue, lymphoid, or other tumor is suspected rather than ovarian cancer. FNA, with or without a core biopsy, is also used to diagnose the recurrence or metastasis of a previously documented ovarian cancer.^{40,41}

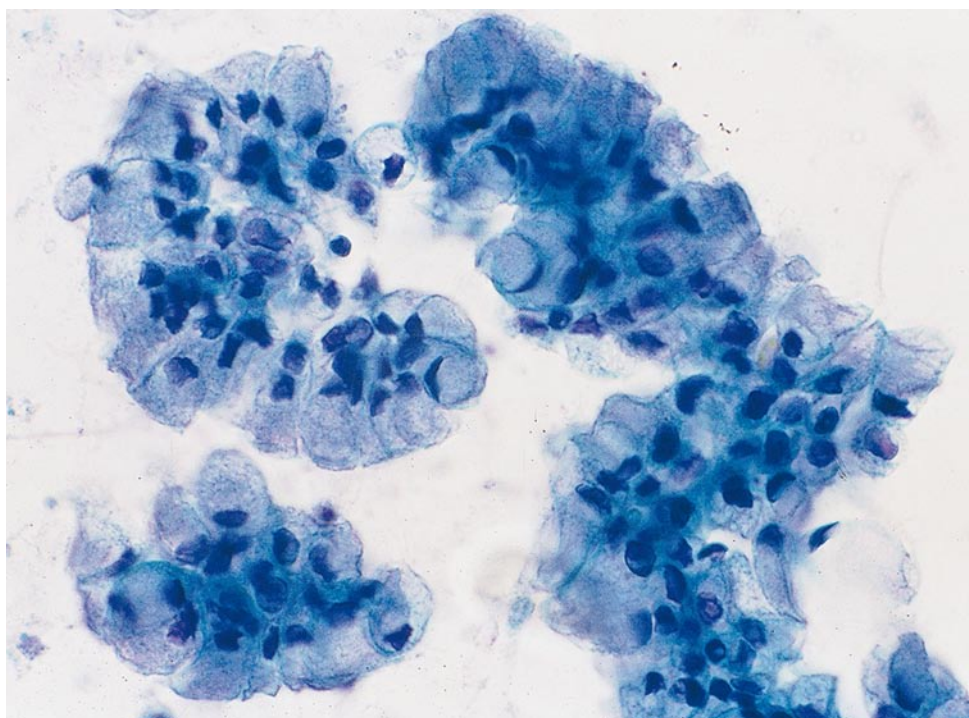


Figure 15.10 Mucinous cystadenoma. Some mucinous cystadenomas have a mixture of endocervical-type cells and intestinal-type goblet cells (Papanicolaou stain).

Serous Borderline Tumor and Adenocarcinoma

The most common malignant ovarian neoplasm is the serous adenocarcinoma. Often bilateral, serous adenocarcinomas commonly contain cystic and solid foci. Histologic samples show pleomorphic cells lining the cystic areas, forming papillary projections and invading the stroma. About 30% contain psammoma bodies.

Like serous adenocarcinomas, serous borderline tumors can be both solid and cystic. Although the degree of nuclear atypia is usually less pronounced in a borderline tumor than in a carcinoma, it is not possible to distinguish between the two cytologically^{27,28}; the diagnosis of adenocarcinoma is based on identifying stromal invasion. Nevertheless, because borderline tumors are generally of lower grade than carcinomas, some generalizations are possible.

CYTOMORPHOLOGY OF SEROUS BORDERLINE TUMOR:

- twisted sheets and spheres
- branching clusters
- mild to moderate nuclear atypia
- large cytoplasmic vacuoles (some cells)
- psammoma bodies
- stripped fibrovascular cores

Aspirates from serous borderline tumors are often sparsely cellular because the needle samples only cyst contents, resulting in a false-negative diagnosis.^{16,22,23} When sampled adequately, the specimen is composed of atypical cells in spheres, branching clusters, and sheets (Fig. 15.11).

CYTOMORPHOLOGY OF SEROUS ADENOCARCINOMA:

- clusters and isolated cells
- large pleomorphic cells
- round nuclei
- prominent nucleoli
- psammoma bodies

Aspirates from serous adenocarcinomas are usually quite cellular, composed of atypical cells in papillary clusters. The nuclei are large and pleomorphic, and nucleoli are prominent (Fig. 15.12). Many atypical bare nuclei are present.³⁹ The cytoplasm, like that of serous borderline tumors, can have large vacuoles. Psammoma bodies accompanied by a rim of malignant cells are seen in some but not all cases.²⁷

Nuclear atypia distinguishes serous borderline tumors and adenocarcinomas from benign serous cystadenomas. Although aspirates of cellular follicle

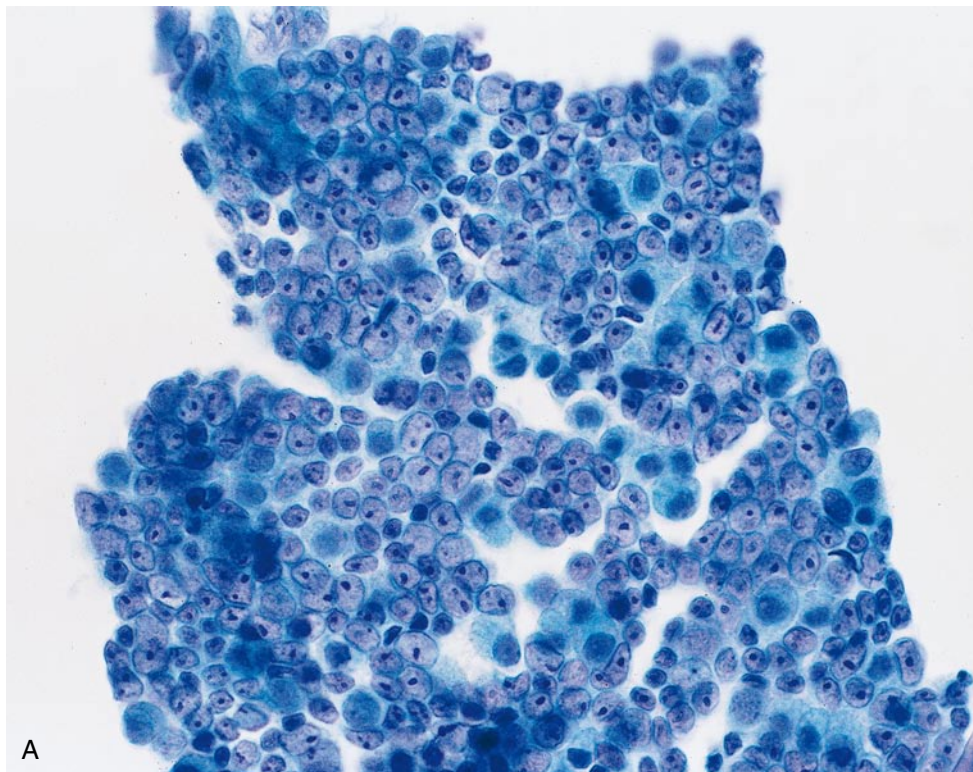


Figure 15.11 Serous borderline tumor. A, The cells are arranged in a crowded sheet. There is mild to moderate atypia.

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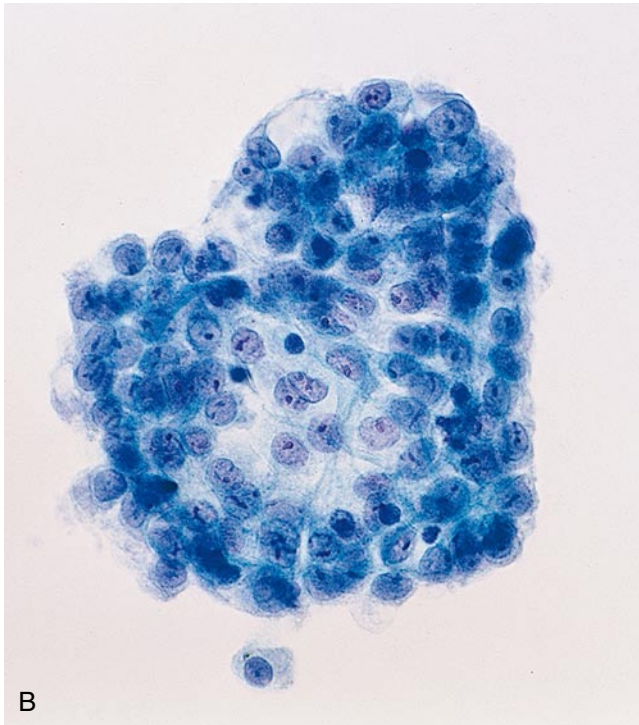


Figure 15.11 (Continued) **Serous borderline tumor.** B, In this tight spherical aggregate, some cells have large cytoplasmic vacuoles. C, Psammoma bodies are a common finding (Papanicolaou stain).

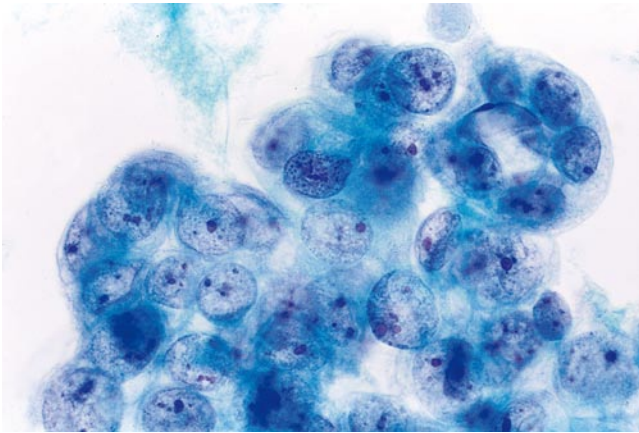


Figure 15.12 **Serous adenocarcinoma.** The malignant cells often have large, round, and pleomorphic nuclei, and nucleoli are prominent (Papanicolaou stain).

cysts, like those of serous adenocarcinomas, can be highly cellular and show mitotic activity, follicle cysts have uniform nuclei and negative sonographic or laparoscopic findings. As mentioned previously, the distinction between a borderline tumor and a serous adenocarcinoma is possible only on the surgically excised specimen. The distinction between serous adenocarcinoma and other epithelial malignancies like mucinous adenocarcinoma and clear cell carcinoma, especially with poorly differentiated tumors, is sometimes difficult.²⁷

Mucinous Borderline Tumor and Adenocarcinoma

Mucinous adenocarcinomas are less common than serous adenocarcinomas. They are usually large, multilocular cysts with solid areas and papillary excrescences. Metastatic intestinal adenocarcinomas to the ovary, particularly from the appendix, are histologically similar to primary mucinous ovarian cancers and need to be excluded by a combination of clinical features, extensive histologic sampling, and immunophenotyping with keratins CK7 and CK20.

CYTOMORPHOLOGY OF MUCINOUS ADENOCARCINOMA:

- columnar mucinous cells with mild atypia or groups of pleomorphic large cells with prominent nucleoli
- cytoplasmic vacuolization
- extracellular mucin
- macrophages

Aspirates from mucinous adenocarcinomas are cellular, composed of isolated cells and cells in sheets or grouped in irregular clusters. Cells from well-differentiated tumors are columnar, contain mucin, and have mild nuclear atypia. The nuclei of poorly differentiated tumors are pleomorphic and indistinguishable from poorly differentiated serous adenocarcinomas; some tumors show a range of differentiation (Fig. 15.13).

As is the case for serous tumors, cytologic samples cannot accurately distinguish a borderline mucinous tumor from a well-differentiated mucinous adenocarcinoma; histologic examination for the presence of invasion is needed. Aspirates from mucinous borderline tumors, as is the case with their serous counterparts, are a common cause of false-negative results.

Spread of a mucinous ovarian tumor into the peritoneum can lead to pseudomyxoma peritonei, but most ovarian tumors associated with pseudomyxoma peritonei are actually metastases from an occult appendiceal primary. Aspirates from pseudomyxoma peritonei are hypocellular, composed predominantly of abundant mucin.⁴² Atypical mucinous glands are identified in some but not all cases.

Endometrioid Carcinoma

Endometrioid carcinoma comprises 10% to 20% of ovarian carcinomas and is bilateral in 28% of cases.³⁷ Up to 42% are associated with endometriosis, and 15% to 20% with a coexisting adenocarcinoma of the endometrium.³⁷ Endometrioid carcinomas are usually cystic and solid tumors with foci of necrosis and hemorrhage. Morphologically similar to the usual type of adenocarcinoma of the endometrium, they are graded using the same criteria. Squamous differentiation may be seen.

CYTOMORPHOLOGY OF ENDOMETRIOID CARCINOMA:

- numerous isolated cells
- strips or crowded glands
- palisading
- elongated columnar shape

Aspirates yield numerous isolated elongated cells, as well as occasional short strips of cells and intact or “broken” glands (Fig. 15.14A). Cell block sections are helpful in revealing the resemblance of the neoplasm to endometrial glands (Fig. 15.14B). The background is usually bloody and contains hemosiderin-laden macrophages. It may be impossible to distinguish an endometrioid carcinoma from other surface epithelial malignancies, such as a serous or mucinous adenocarcinoma, especially with high-grade tumors.^{1,12,27,43}

Clear Cell Carcinoma

Clear cell carcinoma of the ovary resembles the clear cell carcinomas of the endometrium, cervix, and vagina. Tumor cells have large, pleomorphic, often eccentrically placed nuclei with prominent nucleoli. The cytoplasm is abundant and vacuolated or scant and eosinophilic. Within or adjacent to the tumor cell clusters there may be hyaline extracellular material that stains pink-purple with Romanowsky stains.⁴⁴ The background shows necrosis.

GERM CELL TUMORS

Germ cell tumors are most common in the ovary and testis but can also be seen in the retroperitoneum, mediastinum, and the midline of the central nervous system. Neoplasms identical to those of the ovary occur in the testis and extragonadal sites. Germ cell tumors can occur at any age, but they are most common during the reproductive years. They comprise 30% of all ovarian tumors. The vast majority of those seen in adults (up to 95%) are benign cystic teratomas (dermoid tumors). In contrast,

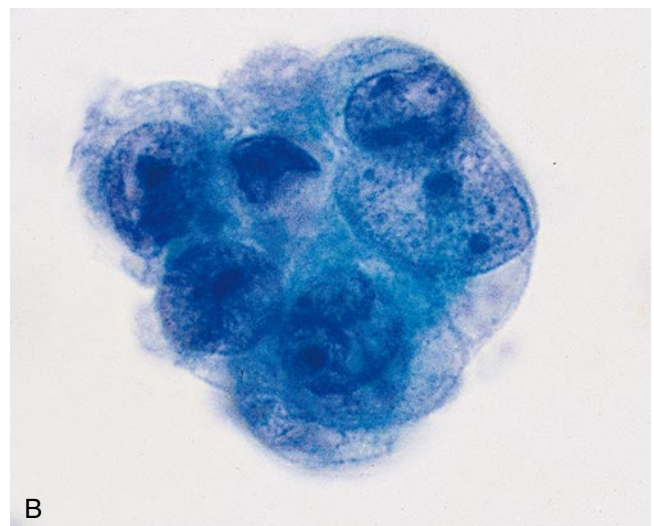
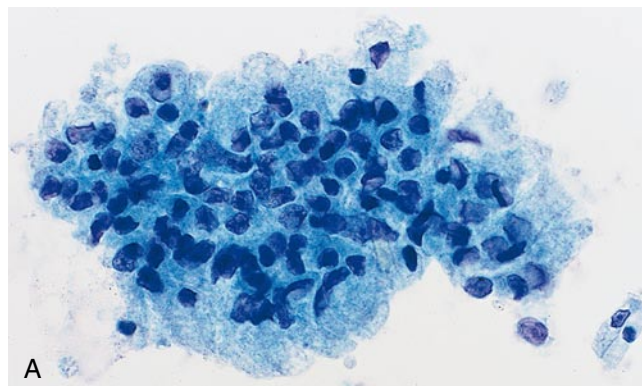


Figure 15.13 Mucinous adenocarcinoma. A, Some sheets of mucinous cells show only mild atypia (compare with Figs. 15.9, 15.10). B, Other cells are markedly atypical, with little if any mucinous differentiation (Papanicolaou stain).

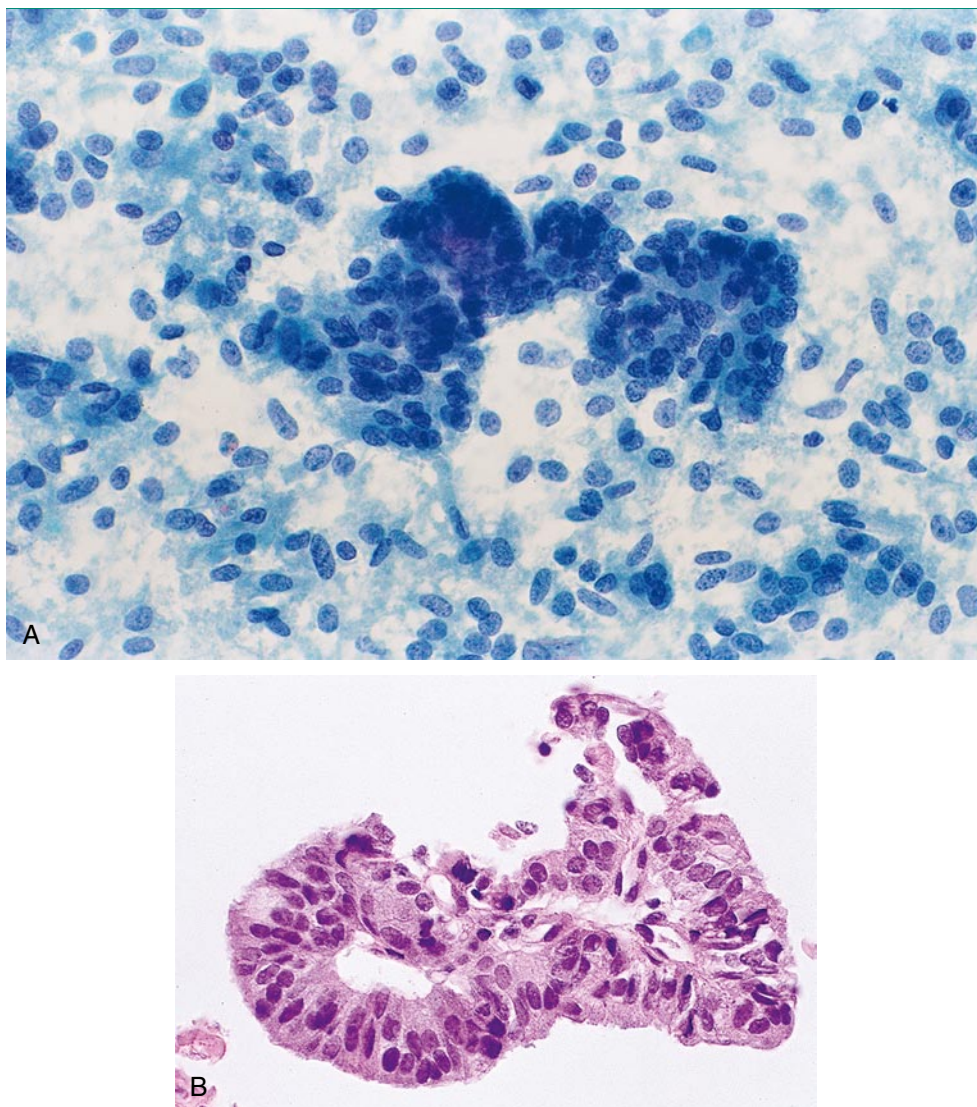


Figure 15.14 Endometrioid adenocarcinoma. A, The cells have elongated nuclei and a narrow columnar shape. Some are arranged in pseudostratified strips and glands (Papanicolaou stain). B, Cell block sections reveal endometrial-like glands with atypia (hematoxylin-eosin [H & E] stain).

up to one third of germ cell tumors in children are malignant.

Although some germ cell tumors are “pure” tumors, such as the pure dysgerminoma, many are composed of a mixture of germ cell subtypes.

Teratoma

Mature Teratoma

Mature teratomas are the most common of the germ cell tumors. Although they are seen at any age, they usually occur during the reproductive years. Most are cystic (mature cystic teratoma or dermoid cyst) and composed of tissue derived from ectoderm, endoderm, and meso-

derm, with ectodermal derivatives such as skin and hair the most common. Sebaceous glands are prominent. Struma ovarii, a variant of mature teratoma, is ectopic thyroid tissue in the ovary. Mature teratomas are rarely aspirated because their sonographic features (particularly the tooth) are diagnostic.

FNA yields cellular material containing predominantly anucleated squamous cells (Fig. 15.15). Ciliated cells, detached ciliary tufts, and mucinous cells are seen in some cases.³⁸ With transvaginal aspirations, contamination of a benign epithelial cyst by normal vaginal squamous cells mimics this picture.⁸ A confident diagnosis of a mature teratoma can be made, however, if some other route was taken to obtain the specimen. In rare cases, a mature cystic teratoma may undergo

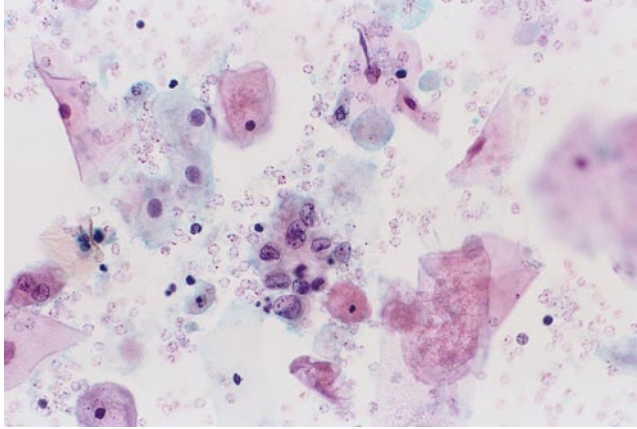


Figure 15.15 Cystic teratoma. Mature squamous cells and occasional glandular cells are present. The squamous cells are indistinguishable from benign vaginal squamous cells, but this sample was obtained percutaneously rather than transvaginally (Papanicolaou stain).

malignant transformation, most commonly to squamous cell carcinoma.

Immature Teratoma

Immature teratomas are rare, malignant, rapidly growing neoplasms, usually solid and unilateral, most commonly in the first two decades of life. These tumors are composed of derivatives from all three germ cell layers and contain a variable amount of immature or embryonal-type tissue. Microscopically, immature or embryonal tissue, usually admixed with benign mature elements, is characteristic of these tumors. Immature neuroectoderm is particularly prominent.

Carcinoid Tumor

Carcinoid tumors of the ovary are usually a component of mature teratomas, but they may also occur in a pure form. It is important to distinguish a primary ovarian tumor from a metastatic carcinoid tumor to the ovary, a distinction that requires clinical correlation and is not possible by cytologic examination. Metastatic carcinoid tumors to the ovary almost always originate from a gastrointestinal primary and are most often bilateral.

FNA reveals numerous isolated cells and loose clusters. Occasionally, rosettes are seen. Tumor cells have an eccentrically placed round or oval nucleus and the chromatin has a granular, “salt-and-pepper” appearance. The cytoplasm is eosinophilic and granular, with distinct outlines. Intracytoplasmic neurosecretory granules are identified by immunocytochemistry for chromogranin and other neuroendocrine markers.

Dysgerminoma

Dysgerminomas constitute 1% to 5% of all malignant ovarian tumors and 40% of all malignant ovarian germ cell tumors. They are often large, solid tumors and are bilateral in 15% to 20% of cases. They are most frequent in women under the age of 30. Because they are highly radiosensitive, accurate diagnosis is crucial so that appropriate therapy may be instituted. Most tumors have rounded or bosselated contours. Large tumors may show cystic areas associated with necrosis and hemorrhage. Histologic examination reveals sheets, nests, and cords of uniform, large, polyhedral tumor cells with centrally placed round nuclei; prominent, sometimes bizarre, eosinophilic nucleoli; and coarsely granular chromatin. The cytoplasm of these cells is abundant, well defined, and eosinophilic or vacuolated. It contains both lipid and glycogen, and stains positively with the periodic acid-Schiff (PAS) reaction. The tumor cells are separated by fibrous septa infiltrated by lymphocytes, some arranged as lymphoid follicles with germinal centers. Granulomas are seen in some cases. A small percentage (3%) contain syncytiotrophoblastic cells that secrete human chorionic gonadotropin.⁴⁵ Ultrastructurally, dysgerminoma cells contain intracytoplasmic annulated lamellae, lipid, and glycogen.⁴⁶

Aspirates from dysgerminomas are usually highly cellular, composed predominantly of isolated tumor cells, with some loose syncytium-like clusters. In the background are small lymphocytes and, in some cases, granulomas. A characteristic “tiger-stripe” background, similar to that seen in seminomas, is seen on air-dried preparations. Necrosis and hemorrhage may be present. The tumor cells have a large, round, centrally placed nucleus with one or more prominent, irregularly shaped nucleoli. The cytoplasm may be clear or granular. Mitoses are present.

Embryonal Carcinoma and Other Malignant Germ Cell Tumors

In contrast with their testicular counterparts, embryonal carcinomas, endodermal sinus tumors, and choriocarcinomas of the ovary are extremely rare. They are aggressive neoplasms that often present at an advanced stage of disease. Histologically, they are primitive large cell neoplasms with marked nuclear anaplasia. Necrosis and hemorrhage are prominent features.

The tumor cells of embryonal carcinoma have a centrally placed, large, round or highly irregular nucleus with several nucleoli. The cytoplasm is indistinct and pale. Bizarrely shaped cells and mitoses are common. Cells of yolk sac tumors resemble those of poorly

differentiated adenocarcinomas. They are cohesive, pleomorphic cells with prominent nucleoli.¹³ Some tumor cells contain intracytoplasmic dense hyaline globules, composed of AFP; others have vacuolated cytoplasm.⁴⁷ Mucoid and basement membrane-like material may be present in the background.⁴⁸ Choriocarcinomas are composed of malignant cytotrophoblast and syncytiotrophoblast. These tumors secrete human chorionic gonadotropin.

A cytologic diagnosis of malignancy in this group of tumors is straightforward, but correct subtyping and distinction from a poorly differentiated epithelial malignancy requires correlation with serologic markers and immunocytochemical studies. Most dysgerminomas show no immunoreactivity for keratin proteins, epithelial membrane antigen (EMA), AFP, or CEA, but are reactive instead for placental alkaline phosphatase. In contrast, embryonal carcinoma, yolk sac tumor, and choriocarcinoma are keratin-positive.

SEX CORD-STROMAL TUMORS

Sex cord-stromal tumors constitute approximately 8% of all ovarian neoplasms and include tumors derived from granulosa cells, theca cells, Sertoli cells, Leydig cells, and the fibroblasts of the ovarian stroma.³⁷ Many of these tumors produce and secrete steroid hormones, which result in clinically apparent estrogenic or, less commonly, androgenic changes.

Granulosa Cell Tumors

Granulosa cell tumors account for about 1.5% of all ovarian tumors. There are two major subcategories: the adult and juvenile types.

Adult Granulosa Cell Tumor

The adult granulosa cell tumor (AGCT) comprises more than 95% of all granulosa cell tumors and occurs in middle aged and postmenopausal women. Typically unilateral, the tumor grows slowly but is capable of metastasis. Many AGCTs secrete estrogens; prolonged elevated levels of estrogen may result in endometrial hyperplasia or carcinoma. Rarely, androgens are secreted, with the resultant signs of virilization. Although about 10% of these tumors are accompanied by ascites, the fluid does not usually contain malignant cells.³³ Although most commonly solid and cystic, with foci of hemorrhage and necrosis, purely solid or cystic tumors are also seen. The tumor is composed of neoplastic granulosa cells arranged in a variety of architectural patterns. Most characteristic is the microfollicular pattern, which shows Call-Exner bodies (i.e., tumor cells arranged around small cavities containing eosinophilic fluid). Granulosa cell tumors are

immunoreactive for alpha-inhibin, CD99, cytokeratin (punctate), calretinin, S-100, and smooth muscle actin and are negative for CK7 and epithelial membrane antigen.

CYTOMORPHOLOGY OF ADULT GRANULOSA CELL TUMOR:

- highly cellular
- isolated cells, sheets, trabeculae, or clusters
- Call-Exner bodies
- small-sized to medium-sized cells
- round nuclei
- nuclear grooves (some cases)
- ill-defined cytoplasm
- globular basement membrane-like material (best with Romanowsky-type stains)

FNA samples are highly cellular. Small-sized to medium-sized cells have a centrally placed, round or oval, monomorphous nucleus (Fig. 15.16A). Nuclear chromatin is pale and finely dispersed. Nuclei are usually round or oval. Nuclear grooves (“coffee bean nuclei”) are easily visible in only a minority (about 20%) of cases.^{41,49,50} Cytoplasm is scant, poorly defined, and pale.³³ Mitoses are rare. Call-Exner bodies are seen in 40% of FNAs.⁵⁰ In some cases, Romanowsky-type stains reveal globular basement membrane-like material.⁴¹ Almost one half of cases show a prominent, arborizing vascular pattern.⁵⁰ Cell block sections are helpful in highlighting one or several of the various growth patterns of granulosa cell tumors: microfollicular, trabecular, insular, watered-silk, and gyriform (Fig. 15.16B).

DIFFERENTIAL DIAGNOSIS OF ADULT GRANULOSA CELL TUMOR:

- cellular follicle cyst
- other sex cord-stromal tumors
- carcinoid tumor
- endometrioid carcinoma
- small cell carcinoma, pulmonary type

The cytomorphology of a cellular follicle cyst (see Figs. 15.1 to 15.3) mimics that of an AGCT.⁵¹ Correlation with sonographic and laparoscopic findings can be key to avoiding an erroneous interpretation; cellular follicle cysts are usually unilocular and clinically entirely benign. Other sex cord-stromal tumors like the Sertoli-Leydig cell tumor and the sex cord tumor with annular tubules cannot be reliably distinguished from an AGCT by cytology or immunohistochemistry.^{40,41,49} A carcinoid tumor lacks nuclear grooves and is immunoreactive for chromogranin. Call-Exner bodies mimic the appearance of endometrioid tubules, but cytokeratin staining is stronger and more diffuse in endometrioid carcinomas.

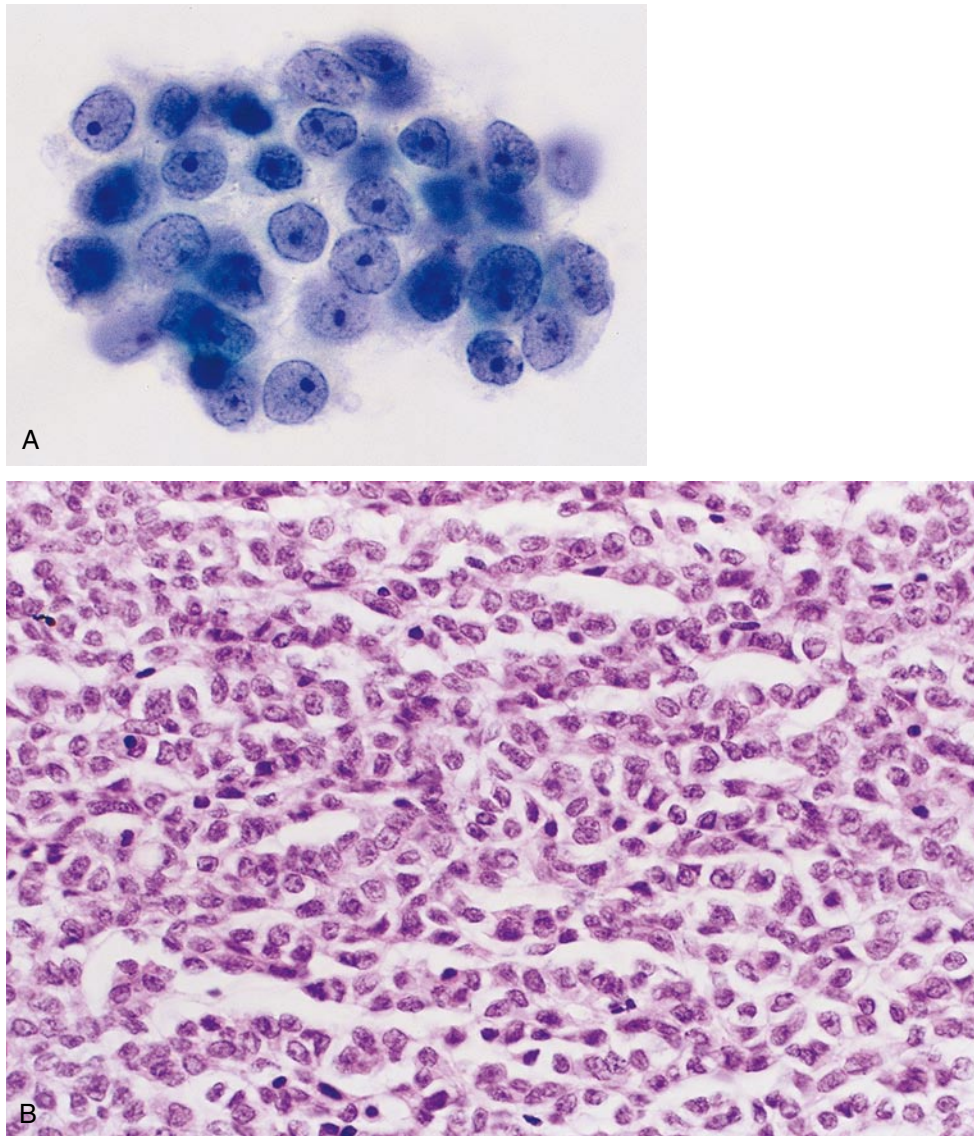


Figure 15.16 Adult-type granulosa cell tumor (AGCT). A, The small-sized to medium-sized cells are arranged in loose clusters. They are difficult to distinguish from normal granulosa cells (compare with Figs. 15.2, 15.3). B, Large fragments of tumor in cell block sections may show one of the characteristic architectural patterns, in this case the watered-silk pattern (hematoxylin-eosin [H & E] stain).

Two rare ovarian tumors are composed partly or completely of undifferentiated small cells: *small cell carcinoma, hypercalcemic type*; and *small cell carcinoma, pulmonary type*.³⁷ The hypercalcemic type is more common in young women and therefore needs to be considered in the differential diagnosis of juvenile granulosa cell tumor. The small cell carcinoma, pulmonary type, resembles small cell carcinoma of the lung (Fig. 15.17). It typically occurs in postmenopausal women and needs to be considered in the differential diagnosis of AGCT.

Juvenile Granulosa Cell Tumor

Juvenile granulosa cell tumors (JGCTs) occur predominantly in childhood and adolescence. Like AGCT, JGCTs secrete estrogens and thus are often associated with sexual pseudoprecocity. JGCTs are usually solid and cystic, unilateral, and restricted to the ovary. Patients may

present with an acute abdomen after rupture of the tumor capsule. The tumor forms follicles lined by granulosa cells and luteinized theca cells. In contrast to AGCT, almost all nuclei lack grooves. Despite the presence of pleomorphism and frequent mitoses, only 10% behave aggressively. The cells of JGCT are positive for cytokeratin, inhibin, and vimentin,⁵² and lack reactivity for CEA, human chorionic gonadotropin, and AFP.

On FNA, tumor cells are seen in loose clusters and single cells.⁵⁰ Nuclei are round, with fine chromatin and small to prominent nucleoli. There may be nuclear protrusions, but nuclear grooves are absent. Some mitoses are seen. There is a moderate amount of granular cytoplasm, and the oil-red-O stain demonstrates intracytoplasmic lipid. Call-Exner bodies are usually not found.

JGCTs should be distinguished from the small cell carcinoma, hypercalcemic type, which occurs in the same age range. The clinical association of JGCT with

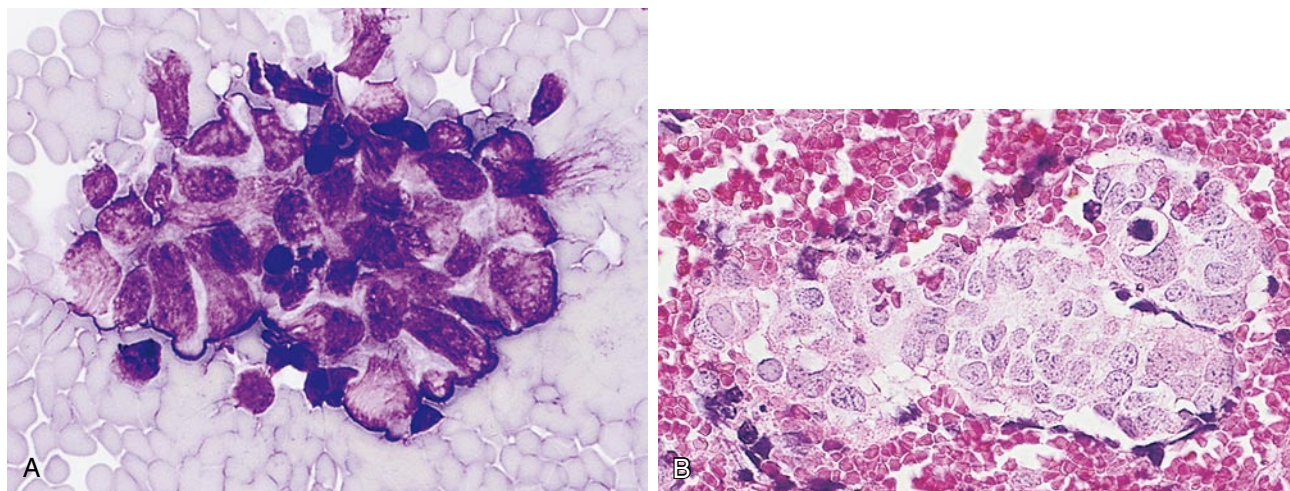


Figure 15.17 Small cell carcinoma of the ovary. This tumor resembles the small cell carcinomas of the lung and other organs. A, Wright-Giemsa-stained smear. B, Hematoxylin-eosin (H & E)-stained cell block section.

estrogenic manifestations, and small cell carcinoma with hypercalcemia are helpful clues. Immunostaining for inhibin (positive in JGCT, negative in small cell carcinoma) can provide confirmation.

Thecoma

Thecomas, benign tumors of theca cells, are more common in postmenopausal women and are usually solid and unilateral tumors. They may be functional, secreting estrogens or androgens, or non-functional. Microscopically, the tumor is composed of monomorphous oval or spindle-shaped cells with cytoplasm that shows variable degrees of luteinization.

Unlike aspirates from granulosa cell and Sertoli-Leydig cell tumors, aspirates from thecomas are usually hypocellular.²⁸ They are composed of isolated elongated cells and loose clusters. The nuclei are spindle shaped, with finely granular chromatin. The cytoplasm is clear and contains numerous lipid vacuoles.

Fibroma

Fibromas are benign, nonfunctional, stromal tumors that comprise 4% of ovarian neoplasms. They are more common during reproductive years. These tumors may be accompanied by ascites and pleural effusion (Meigs' syndrome). Histologically, fibromas are composed of bundles of spindle-shaped cells arranged in a whorling or storiform pattern. The cells have a fibroblastic appearance, and collagen is usually abundant. Intracellular edema and intracytoplasmic lipid are sometimes observed.

Aspirates are hypocellular and contain bundles of fibroblastic spindle-shaped cells similar to those seen in thecomas. The distinction between a fibroma and a thecoma by FNA cytology is impossible because luteinized cells can be a component of both tumors.

UNCOMMON PRIMARY OVARIAN TUMORS

Benign and malignant mesenchymal tumors, bone-producing and cartilage-producing tumors, vascular tumors, and primary lymphomas of the ovary are rare, as are tumors of neural origin and those derived from the mesothelium (adenomatoid tumors). Leiomyomas may have prominent cystic degeneration; aspirates from such tumors contain benign-appearing foam cells suggestive of a benign cyst. When leiomyomas are not cystic, the aspirates are usually scant and contain only occasional smooth muscle cells. Aspirates from lymphomas yield numerous isolated cells that are usually easily recognizable as lymphoid in origin.

METASTATIC TUMORS

The most common tumors that metastasize to the ovaries arise in the urogenital tract, colon, stomach, and breast. Approximately 15% to 20% of bilateral ovarian malignancies are metastatic. Tumors that occur as multiple nodules on the ovarian surface are likely to be metastatic.

Krukenberg tumors are characterized by mucin-filled signet ring-shaped cells metastatic to the ovary.²⁸ The majority of these tumors arise in the stomach, but tumors of the colon, appendix, and breast also cause this pattern of spread.

In many cases, distinguishing between a primary ovarian carcinoma and a metastasis is impossible by cytomorphology alone. Measuring the concentration of tumor-associated antigens in cyst fluid is useful; unlike ovarian carcinomas, metastatic colon cancers have a high CEA level combined with a low CA-125 level.¹⁷

REFERENCES

- De Crespigny L, ChRobinson HP, Davoren RA, Fortune D: The "simple" ovarian cyst: Aspirate or operate? *Br J Obstet Gynecol* 1989;96:1035-1039.
- DePriest PD, Shenson D, Fried A, et al.: A morphology index based on sonographic findings in ovarian cancer. *Gyn Oncol* 1993;51:7-11.
- Yee H, Greenebaum E, Lerner J, et al.: Transvaginal sonographic characterization combined with cytologic evaluation in the diagnosis of ovarian and adnexal cysts. *Diagn Cytopathol* 1994;10:107-112.
- Rubenchik I, Auger M, Casper RF: Fine-needle aspiration cytology of ovarian cysts in in vitro fertilization patients: A study of 125 cases. *Diagn Cytopathol* 1996;15(4):341-344.
- Guariglia L, Conte M, Are P, Rosati P: Ultrasound-guided fine-needle aspiration of ovarian cysts during pregnancy. *Eur J Obstet Gynecol Rep Biol* 1999;82:5-9.
- Allias F, Chanoz J, Blache G, et al.: Value of ultrasound-guided fine-needle aspiration in the management of ovarian and paraovarian cysts. *Diagn Cytopathol* 2000;22:70-80.
- Mulvany N, Ostor A, Teng G: Evaluation of estradiol in aspirated ovarian cystic lesions. *Acta Cytol* 1995;39:663-668.
- Greenebaum E, Mayer JR, Stangel JJ, Hughes P: Aspiration cytology of ovarian cysts in in vitro fertilization patients. *Acta Cytol* 1992;36:11-18.
- Stanley MW, Horwitz CA, Frable WJ: Cellular follicular cyst of the ovary: Fluid cytology mimicking malignancy. *Diagn Cytopathol* 1991;7:48-52.
- Abrams J, Silverberg SG: The role of intraoperative cytology in the evaluation of gynecologic disease. *Pathol Ann* 1989;24 (part 2): 167-187.
- Ganji P, Nadi M: Aspiration cytology of ovarian neoplasms: A review. *Acta Cytol* 1984;28:329-332.
- Kjellgren O, Angstrom T, Bergman E, Wiklund DE: Fine-needle aspiration biopsy in diagnosis, and classification of ovarian carcinoma. *Cancer* 1971;28:967-976.
- Mulvany NJ: Aspiration cytology of ovarian cysts and cystic neoplasms: A study of 235 aspirates. *Acta Cytol* 1996;40:911-920.
- Ganji P: Fine-needle aspiration cytology of the ovary. *Clin Lab Med* 1995;15(3):705-726.
- Trimbos JB, Hacker NF: The case against aspirating ovarian cysts. *Cancer* 1993;72:828-831.
- Geier GR, Strecker JR: Aspiration cytology and E2 content in ovarian tumors. *Acta Cytol* 1981;25:400-406.
- Pinto MM, Bernstein LH, Brogan DA, et al.: Measurement of CA125, carcinoembryonic antigen, and alpha-fetoprotein in ovarian cyst fluid: Diagnostic adjunct to cytology. *Diagn Cytopathol* 1990;6:160-163.
- Wojcik EM, Selvaggi SM: Fine-needle aspiration cytology of cystic ovarian lesions. *Diagn Cytopathol* 1994;11:9-14.
- Tahir Z, Yusuf NW, Ashraf M, et al.: Fine needle aspiration of unilocular ovarian cysts—A cytohistological correlation. *J Pak Med Assoc* 2004;54(5):266-269.
- Martinez-Onsurbe P, Ruiz Villaespesa A, Sanz Anquela JM, Valenzuela Ruiz PL: Aspiration cytology of 147 adnexal cysts with histologic correlation. *Acta Cytol* 2001;45(6):941-947.
- Volmar KE, Herman CM, Creager AJ: Fine-needle aspiration cytology of endometrioid adenofibroma of the ovary. *Diagn Cytopathol* 2004;31(1):38-42.
- Granados R: Aspiration cytology of ovarian tumors. *Curr Opin Obstet Gynecol* 1995;7:43-48.
- Moran O, Menczer J, Ben-Barauch G, et al.: Cytologic examination of ovarian cyst fluid for the distinction between benign and malignant tumors. *Obstet Gynecol* 1993;82:444-446.
- Diernaes E, Rasmussen J, Soerensen T, Hasch E: Ovarian cysts: management by puncture? *Lancet* 1987;1:1084.
- Nuñez C, Diaz JI: Ovarian follicular cysts: A potential source of false positive diagnoses in ovarian cytology. *Diagn Cytopathol* 1992;8:532-536.
- Selvaggi SM: Fine-needle aspiration cytology of ovarian follicle cysts with cellular atypia from reproductive-age patients. *Diagn Cytopathol* 1991;7:189-192.
- Ganji P, Dickinson B, Harrison T, et al.: Aspiration cytology of neoplastic and non-neoplastic ovarian cysts: Is it accurate? *Int J Gynecol Pathol* 1996;15(2):94-101.
- Uguz A, Ersoz C, Bolat F, et al.: Fine needle aspiration cytology of ovarian lesions. *Acta Cytol* 2005;49(2):144-148.
- Pinto MM, Greenebaum E, Simsir A, et al.: CA-125 and carcinoembryonic antigen assay versus cytodagnostic experience in the classification of benign ovarian cysts. *Acta Cytol* 1997;41:1456-1462.
- Ramzy I, Delaney M, Rose P: Fine needle aspiration of ovarian masses: II. Correlative cytologic and histologic study of nonneoplastic cysts and noncelomic epithelial neoplasms. *Acta Cytol* 1979;23:185-193.
- Kovacic J, Rainer S, Levincnik A, Cizelj T: Cytology of benign ovarian lesions in connection with laparoscopy. In Zajicek J (ed) *Aspiration Biopsy Cytology, Part 2: Cytology of Infradiaphragmatic Organs*. Basel, S. Karger, 1979.
- Tao LC (ed): *Transabdominal Fine-Needle Aspiration Biopsy*. New York, Igaku-Shoin, 1990.
- Ehya H, Lang WR: Cytology of granulosa cell tumor of the ovary. *Am J Clin Pathol* 1986;85:402-405.
- Selvaggi SM: Cytology of nonneoplastic cysts of the ovary. *Diagn Cytopathol*. 1990;6:77-85.
- Tabbara SO, Nayar R: Fluid cytology in nonneoplastic ovarian cysts [Abstract]. *Acta Cytol* 1994;38:833-834.
- Ballouk F, Ross JS, Wolf BC: Ovarian endometriotic cysts: an analysis of cytologic atypia and DNA ploidy patterns. *Am J Clin Pathol* 1994;102:415-419.
- Tavassoli FA, Devilee P (eds): *World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs*. Lyon, France, IARC Press, 2003.
- Rivasi F, Gasser B, Morandi P, Philippe E: Ciliated bodies in ovarian cyst aspirates. *Acta Cytol* 1993;37:489-493.
- Ramzy I, Delaney M: Fine needle aspiration of ovarian masses: I. Correlative cytologic and histologic study of celomic epithelial neoplasms. *Acta Cytol* 1979;23:97-104.
- Watson B, Siegel CL, Ylagan LR: Metastatic ovarian Sertoli-cell tumor: FNA findings with immunohistochemistry. *Diagn Cytopathol* 2003;29(5):283-286.
- Ylagan LR, Middleton WD, Dehner LP: Fine-needle aspiration cytology of recurrent granulosa cell tumor: Case report with differential diagnosis and immunocytochemistry. *Diagn Cytopathol* 2002;27(1):38-41.
- Leiman G, Goldberg R: Pseudomyxoma peritonei associated with ovarian mucinous tumors: Cytologic appearance in five cases. *Acta Cytol* 1992;36:299-304.
- Nagai S, Nozawa S, Kurihara S, Mukai M: Cytologic and biologic studies of endometrioid carcinoma of the ovary. *Acta Cytol* 1983;27:676-682.
- Atahan S, Ekin C, Icli F, Erdogan N: Cytology of clear cell carcinoma of the female genital tract in fine needle aspirates and ascites. *Acta Cytol* 2000;44(6):1005-1009.
- Young RH: New and unusual aspects of ovarian germ cell tumors. *Am J Surg Pathol* 1993;17:1210-1224.
- Hees K, de Jonge JPA, von Kortzfleisch DHJ: Dysgerminoma of the ovary: Cytologic, histologic, and electron microscopic study of a case. *Acta Cytol* 1991;35:341-344.

47. Nadji M, Sevin BU: Pelvic fine needle aspiration cytology in gynecology. In Linsk JA (ed): Clinical Aspiration Cytology, 2nd ed. Philadelphia, Lippincott, 1989.
48. Akhtar M, Ali MA, Sackey K, et al.: Fine-needle aspiration biopsy diagnosis of endodermal sinus tumor: Histologic and ultrastructural correlations. *Diagn Cytopathol* 1990;6:184-192.
49. Nguyen GK, Redburn J: Aspiration biopsy of granulosa-cell tumor of the ovary: Cytologic findings and differential diagnosis. *Diagn Cytopathol* 1992;8:253-257.
50. Lal A, Bourtsos EP, Nayar R, DeFrias DV: Cytologic features of granulosa cell tumors in fluids and fine needle aspiration specimens. *Acta Cytol* 2004;48(3):315-320.
51. Dejmek A: Fine needle aspiration cytology of an ovarian luteinized follicular cyst mimicking a granulosa cell tumor. A case report. *Acta Cytol* 2003;47(6):1059-1062.
52. Stamp GW, Krausz T: Fine needle aspiration cytology of a recurrent juvenile granulosa cell tumor. *Acta Cytol* 1988;32:533-539.

Soft Tissue

XIAOHUA QIAN

SPECIMEN COLLECTION AND PREPARATION

ANCILLARY STUDIES

REPORTING TERMINOLOGY

ADIPOCYTIC OR LIPOGENIC NEOPLASMS

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Hibernoma
Spindle Cell Lipoma and Pleomorphic Lipoma
Well-Differentiated Liposarcoma or Atypical Lipomatous Tumor
Pleomorphic Liposarcoma

MYXOID NEOPLASMS

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Myxofibrosarcoma, Low and High Grade
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Low-Grade Fibromyxoid Sarcoma
Myxoid or Round Cell Liposarcoma
Myxofibrosarcoma-Like Dedifferentiated Liposarcoma
Lipoblastoma
Chordoma
Extraskeletal Myxoid Chondrosarcoma

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Leiomyosarcoma
Schwannoma
Malignant Peripheral Nerve Sheath Tumor
Synovial Sarcoma
Solitary Fibrous Tumor
Fibromatoses
Dermatofibrosarcoma Protuberans
Inflammatory Myofibroblastic Tumor
Hemangiopericytoma and Fibrosarcoma

FIBROHISTIOCYTIC NEOPLASMS

Giant Cell Tumor of Tendon Sheath, Localized and Diffuse Types
Angiomatoid Fibrous Histiocytoma
(Angiomatoid Malignant Fibrous Histiocytoma)

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Extrasosseous Ewing Sarcoma/Primitive Neuroectodermal Tumor
Desmoplastic Small Round Cell Tumor
Embryonal Rhabdomyosarcoma
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Epithelioid Sarcoma
Clear Cell Sarcoma (Malignant Melanoma of Soft Tissues)
Alveolar Soft Part Sarcoma
Epithelioid Hemangioendothelioma
Epithelioid Angiosarcoma
Granular Cell Tumor

PLEOMORPHIC NEOPLASMS

Undifferentiated High-Grade Pleomorphic Sarcoma (Pleomorphic Malignant Fibrous Histiocytoma)
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NON-NEOPLASTIC SOFT TISSUE LESIONS

Idiopathic Retroperitoneal Fibrosis
Nodular Fasciitis
Elastofibroma
Amyloidoma (Tumoral Amyloidosis)

Few areas in cytology arouse such passionate discourse as fine-needle aspiration (FNA) of soft tissue masses. Critics jump at the opportunity to point out the limitations of the technique but pepper their few valid arguments with outdated fallacies and academic concerns that do not necessarily consider the best interest of individual patients.

One undeniable difficulty with soft tissue FNA is the lack of experience most cytopathologists have with the greater than 130 different soft tissue lesions, including more than 30 types of sarcoma. Because the morphologic heterogeneity is so astounding, it has been suggested

that at least five soft tissue lesions ought to be sampled weekly to maintain practitioner experience.^{1,2} With sarcomas constituting only 0.6% of all malignancies in the United States, this recommendation is unrealistic for most cytopathologists.³ Although restricting FNA of soft tissue lesions to large specialty centers might be desirable, most patients are first seen in community practices. A systematic approach to differential diagnosis, specimen triage, recommendations, and referrals can enhance the efforts of the cytopathologist, regardless of their level of experience.^{1,2,4-7} For this reason, this chapter

focuses on pattern recognition and is organized into sections on adipocytic, myxoid, spindle-cell, fibrohistiocytic, round-cell, epithelioid, and pleomorphic neoplasms.

Sampling error is a problem FNA shares with core and even incisional biopsies. Ancillary techniques like immunohistochemistry and cytogenetics are often indispensable for definitive classification. Concern over needle tract seeding, stemming from the era of larger needles, however, is unfounded.^{1,5,6,8-13}

FNA is a useful screening tool for soft tissue masses. It is the least invasive method for sampling a heterogeneous lesion, does not compromise tissue planes for a subsequent excision, and is cost-effective.^{1,2,5,6,8-10,14-17} Although not widely accepted by most soft tissue pathologists as the sole sampling modality for the primary classification of sarcomas, it plays an important role in triaging patients—the initial clinical differential diagnosis for many soft tissue masses is broad and might include lymphoma and even metastatic carcinoma. FNA can be indispensable as the initial step toward narrowing the possibilities. FNA is especially valuable for confirming a recurrence or metastasis.^{6,14,18-20}

The location, size, and degree of invasiveness of a lesion, and its relationship to surrounding structures are important clues to correct diagnosis. Benign lesions are generally small, circumscribed, and cutaneous or superficial masses, whereas malignant lesions are more often large (>5 cm), infiltrative, and deep-seated (intermuscular or intramuscular, or retroperitoneal).^{1,2,4,6-8,14,21}

Advantages of fine-needle aspiration for soft tissue lesions:

- technical ease
- less invasive; less morbidity
- allows for immediate assessment of specimen adequacy and allocation of tissue for special studies
- no contamination of tissue planes
- cost effective

Most (87% to 100%) cases are correctly diagnosed as benign or malignant, with an average sensitivity and specificity for malignancy of approximately 95%.^{1,2,4,8,9,14,18,22} The distinction between lymphoma, carcinoma, and sarcoma has specificity rates for sarcoma of 54% to 98% and a positive predictive value of 91% to 99%.^{9,17,18,23} The three main reasons for a false diagnosis are sampling error, a technically limited specimen, and misinterpretation.² The false-positive rate for soft tissue FNA is relatively low (0% to 5%). False-negative rates vary a little more (2% to 15%), but any clinically suspicious lesion should be further evaluated by biopsy.^{1,2,4,8,9,18,19}

Rapid evaluation of specimen adequacy at the time of the procedure, with triage of tissue for ancillary studies, decreases the rate of inadequate specimens.^{1,2,5,6,8,14}

Summary: statistical performance characteristics of soft tissue fine-needle aspiration:

- sensitivity rates: as high as 95%
- specificity for sarcoma: 54% to 98%
- false-positive rate: 0% to 5%
- false-negative rate: 2% to 15%

SPECIMEN COLLECTION AND PREPARATION

Cytomorphologic evaluation should be based on both alcohol-fixed, Papanicolaou-stained preparations (for nuclear details) and air-dried, Romanowsky-type stained preparations (for cytoplasmic details and matrix material). Thinlayer preparations offer good nuclear detail, optimize results from aspirates carried out without the benefit of rapid evaluation, and can be used for adjunct studies, but cells can appear smaller, rounder, and falsely epithelioid, and useful background information (e.g., vascular patterns) is sometimes lost.^{1,5,8,22,24-27} Cell block preparations are especially helpful for immunohistochemical studies and for mini-tissue architecture. A concurrent core biopsy taken with a larger gauge needle is commonly obtained when imaging characteristics or the rapid, on-site evaluation of smears suggests a soft tissue tumor.

Specimen collection and preparation:

- use 22- to 25-gauge needles
- separate passes for ancillary studies
- alcohol-fixed, Papanicolaou-stained smears for nuclear detail
- air-dried, Romanowsky-type -stained smears for cytoplasmic and matrix details
- limited use for thinlayer preparations

ANCILLARY STUDIES

Ancillary techniques like immunohistochemistry, cytogenetics, and molecular genetics are often indispensable for the classification of soft tissue lesions. Electron microscopy is only occasionally used for assistance in classifying a poorly differentiated neoplasm, small round blue cell tumor, or soft tissue tumor that exhibits specific ultrastructural features, like the Weibel-Palade bodies of vascular tumors.^{1,28-31} Cell block sections are preferred for immunohistochemical studies, but air-dried or alcohol-fixed direct smears and cytocentrifuge or thinlayer preparations can also be used. Flow cytometry is useful when lymphoma is in the differential diagnosis. Ideally, one makes a dedicated pass for each desired ancillary study.^{9,12,30,32,33}

An increasing number of reproducible, relatively specific cytogenetic abnormalities can be identified (Table 16.1) with conventional chromosomal analysis (karyotyping), fluorescence in-situ hybridization (FISH), and reverse transcriptase-polymerase chain reaction (RT-PCR) techniques.^{4,9,13,34,35} Conventional chromosomal analysis offers the advantages of a full karyotype, but this is counterbalanced by low sensitivity and a longer turnaround time than molecular methods like FISH.^{4,34,36}

FISH accurately labels targeted chromosomal regions, and many cytologic preparations like cytocentrifuge, thinlayer slides, and smears are ideal substrates for FISH because they contain intact cells and nuclei free of sectioning artifact and truncation. When the diagnostic chromosomal aberration is known for a suspected soft tissue tumor, FISH is preferred because the number of cells needed is far less than for conventional karyotyping.^{1,37} Because only a specified chromosomal

TABLE 16.1 CYTOGENETIC ALTERATIONS IN SOFT TISSUE LESIONS

Lesion	Cytogenetic Change(s)	Diagnostic FISH Probe(s)*
Alveolar soft part sarcoma	der(X)t(X;17)(p11;q25)	TFE3
Angiomatoid fibrous histiocytoma	t(12;16)(q13;11)	FUS
	t(12;22)(q13;12)	EWS-R1
	t(2;22)(q34;q12)	EWS-R1
Chondrosarcoma, extraskeletal myxoid	t(9;22)(q22;q12)	EWS-R1
	t(9;17)(q22;q11)	—
	t(9;17)(q22;q11)	—
Clear cell sarcoma	t(12;22)(q13;q12)	EWS-R1
	t(2;22)(q33;q12)	EWS-R1
Dermatofibrosarcoma protuberans	Ring forms of chromosomes 17 and 22	—
	t(17;22)(q21;q13)	—
Desmoid fibromatosis	Trisomy 8 and 20	CEP8/CEP20
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	EWS-R1
Epithelioid sarcoma	t/der(22)(q11.2)	—
Ewing sarcoma/primitive neuroectodermal tumor	t(11;22)(q24;q12)	EWS-R1
	t(21;22)(q12;q12)	EWS-R1
	t(2;22)(q33;q12)	EWS-R1
	t(7;22)(p22;q12)	EWS-R1
	t(17;22)(q12;q12)	EWS-R1
	inv(22)(q12q12)	—
Giant cell tumor of tendon sheath/PVNS	t(1;2)(p13;q37)	—
Hibernoma	11q13 rearrangement	—
Inflammatory myofibroblastic tumor	2p23 rearrangement	ALK
Infantile fibrosarcoma	t(12;15)(p13;q26)	ETV6
Lipoblastoma	8q12 rearrangement or polysomy 8	—
Lipoma, ordinary	12q15 rearrangement	HMGA2
Lipoma, spindle cell and pleomorphic	Deletions of 13q and 16q	—
Liposarcoma, well-differentiated or dedifferentiated	Giant marker chromosomes and ring form of chromosome 12	HMGA2/MDM2
Liposarcoma, myxoid or round cell	t(12;16)(q13;p11)	CHOP (DDIT3) or FUS
	t(12;22)(q13;q12)	CHOP (DDIT3) or EWS
Low-grade fibromyxoid sarcoma	t(7;16)(q34;p11)	FUS
Neuroblastoma (poor prognosis)	Amplification (MYCN)	MYCN
	Deletion of 1p	LSI1p36
	Deletion of 11q	MLL
Rhabdomyosarcoma, alveolar	t(2;13)(q35;q14)	FKHR (FOXO1A)
	t(1;13)(p36;q14), double minutes	FKHR (FOXO1A)
Rhabdomyosarcoma, embryonal	Trisomies 2q, 8, and 20	—
	Loss of heterozygosity at 11p15	—
Schwannoma	Deletion of 22q	—
Synovial sarcoma	t(X;18)(p11;q11)	SYT (SS18)

*www.genenames.org

abnormality is targeted, however, a negative result provides minimal information.^{34,36}

REPORTING TERMINOLOGY

As with other cytologic specimens, the use of general category headings on the report (e.g., benign, atypical, suspicious, positive, non-diagnostic), along with a descriptive interpretation, is useful for clarity of communication. When the findings are not conclusive for a specific entity, a descriptive interpretation with differential diagnosis is appropriate.^{1,4,6,9,18,38}

The most important prognostic consideration with soft tissue tumors is the histologic grade, and some are amenable to grading by FNA.³⁹ Grading should only be attempted when a specific diagnosis can be unequivocally established and the tumor is gradable based on cellularity, pleomorphism, mitoses, and necrosis. A two-tiered grading system of low versus high is probably sufficient in the majority of cases.^{1,2,19,39,40} Low-grade lesions have mild nuclear atypia, minimal or absent necrosis, low cellularity with minimal nuclear overlap, and rare or absent mitoses (<3 per 10 high-power fields). High-grade lesions have moderate to marked nuclear atypia, intermediate to high cellularity with conspicuous to prominent nuclear overlap, definite necrosis, and frequent mitoses.^{19,40}

The presence or absence of mitoses and necrosis is an objective finding worthy of separate mention in the report. A mitotic count based on field unit is rarely possible on cytologic preparations. A statement such as “mitoses are absent” or “mitoses are numerous” suffices to avoid falsely high or low counts.^{19,39}

Reporting soft tissue fine-needle aspiration results:

- general category
- histologic subtype or description with differential diagnosis
- grade
 - mitoses
 - necrosis
- ancillary study results
- notes and recommendations

ADIPOCYTIC OR LIPOGENIC NEOPLASMS

Neoplasms with lipogenic differentiation occur over a broad age range, but malignant tumors occur almost exclusively in adults. Lesions with well-developed adipocytic morphology are difficult to classify accurately.⁴¹ Some authors discourage the use of FNA for large, deep-seated,

intramuscular or retroperitoneal lesions that radiographically appear predominantly fatty.⁹ The diagnosis of a well-differentiated liposarcoma can be missed if the (focal) diagnostic areas are not sampled, and this has led to the suggestion that potentially lipogenic lesions need to be excised and thoroughly sampled for definitive diagnosis. This approach does not take into consideration newer therapeutic options (tumors pretreated before surgery) or the value of ancillary studies (e.g., cytogenetics), which are useful in the classification of most fatty lesions (see Table 16.1).^{4,7-9,42}

Lipoma

Benign lipomas, occurring mainly in adults over the age of 30 years, are common and account for about half of all soft tissue tumors. Most lesions are slowly growing, subcutaneous or intramuscular tumors that occur over a wide anatomic distribution but spare the hands and feet, rarely exceed 10 cm in size, and present as soft, painless lumps. Local recurrence occurs in 1% to 2% of cases.

CYTOLOGY OF LIPOMA:

- small tissue fragments
- large, univacuolated adipocytes of uniform size
- small, bland nuclei without atypia

Smears are composed of small tissue fragments; isolated cells are rarely present. Larger fragments contain thin capillaries, and striated or atrophic muscle fibers can be seen in juxtamuscular or intramuscular tumors. The large, univacuolated adipocytes are of uniform size and shape (Fig. 16.1). Their nuclei are small, round and regular, with evenly distributed chromatin.^{24,43} Scattered foamy macrophages with ingested lipid (called “lipophages”) are common in tumors with regressive changes (Fig. 16.2).

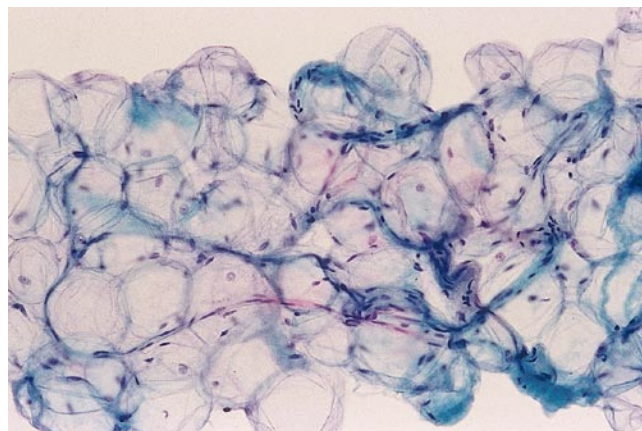


Figure 16.1 Lipoma. Benign lipomas have occasional capillaries, but they are not as numerous as in hibernomas (Papanicolaou stain).

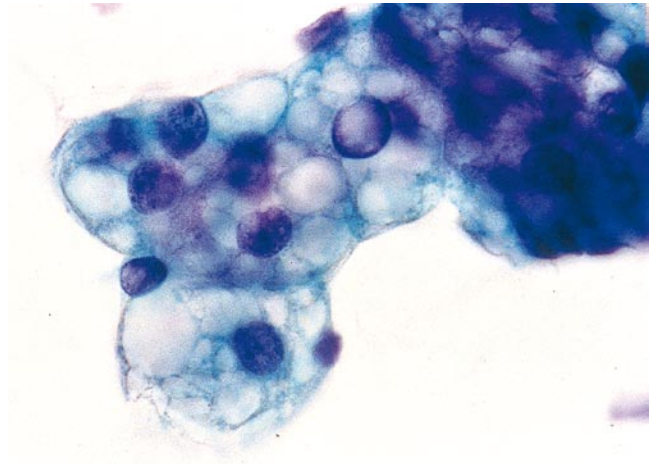


Figure 16.2 Fat necrosis. Multivacuolated histiocytes accompanying areas of fat necrosis or regressive change lack the more distinct, larger cytoplasmic vacuoles, well-demarcated cytoplasmic membranes, and scalloped nuclei of true lipoblasts (Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF LIPOMA:

- subcutaneous adipose tissue
- pleomorphic lipoma
- well-differentiated liposarcoma

Normal subcutaneous adipose tissue lacks the mass-like clinical presentation of a lipoma, thus the distinction between the two is most confidently achieved when the cytopathologist performs the aspiration. Atrophic muscle fibers in some lipoma aspirates can mimic the

floret cells of a pleomorphic lipoma, but atypical mononucleated stromal cells are absent. Lipomas also lack the variable cell size and atypical stromal cells of well-differentiated liposarcoma.^{4,11,24,43,44}

Hibernoma

The hibernoma is a rare benign tumor of brown fat that occurs predominantly in adults aged 20 to 50 years. Reflecting normal brown fat distribution, hibernomas are found in the neck, shoulder, upper back, thigh, mediastinum, and retroperitoneum. Although mostly subcutaneous, they can be intramuscular.

CYTOMORPHOLOGY OF HIBERNOMA:

- small, bland nuclei
- three cell types:
 - mature adipocytes
 - hibernoma cells (with multiple small, uniform cytoplasmic vacuoles)
 - lipoblast-like cells
- many delicate capillaries

Hibernomas yield moderately cellular aspirates of fatty tissue fragments composed of three intermingled elements: (1) mature, univacuolated adipocytes; (2) large, rounded, finely multivacuolated cells with granular cytoplasm (hibernoma cells); and (3) large cells with two or more larger fat vacuoles (lipoblast-like cells). All three cell types have small, uniform, eccentric nuclei. Many delicate capillaries are seen within the fragments (Fig. 16.3).

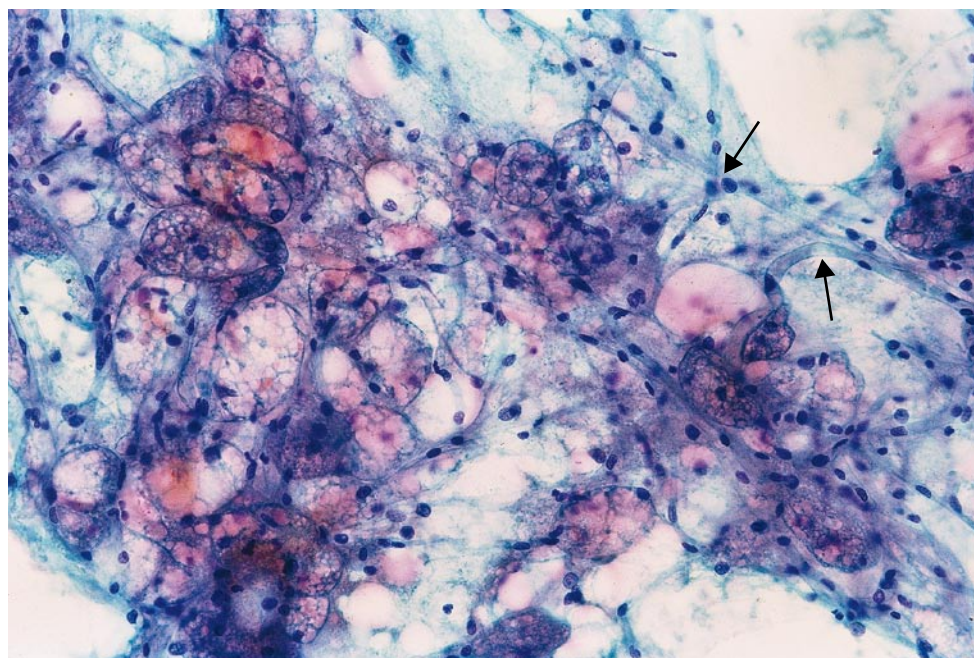


Figure 16.3 Hibernoma. Delicate capillaries (arrows) can be numerous and are closely associated with tumor cells that have distinct granular and multivacuolated cytoplasm. (Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF HIBERNOMA:

- well-differentiated liposarcoma or atypical lipomatous tumor
- myxoid or round cell liposarcoma
- chondroid lipoma
- lipoma with lipophages
- granular cell tumor
- adult rhabdomyoma
- pilosebaceous units

Although lipoblast-like cells are seen in a hibernoma, the atypical lipoblasts characteristic of liposarcomas and the atypical lipomatous tumor are not present. Capillary vessels can be numerous in hibernomas, but they are less prominent than in a myxoid or round cell liposarcoma. The myxoid stroma and chondromyxoid matrix of a chondroid lipoma are not prominent in a hibernoma. Lipophages in a lipoma generally have a larger, plumper nucleus, and their fat vacuoles are more variable in size (see Fig. 16.2). Granular cell tumors lack adipocytic differentiation, and rhabdomyomas have larger cells with dense, fibrillary cytoplasm.⁴³⁻⁴⁵ Normal pilosebaceous units can contaminate any FNA that traverses hair-bearing skin. They are recognized by the admixture of finely vacuolated sebaceous cells with duct-like epithelial cells that have non-vacuolated cytoplasm.

Spindle Cell Lipoma and Pleomorphic Lipoma

Spindle cell and pleomorphic lipomas are benign lipoma variants with overlapping clinical, morphologic, and cytogenetic features; they likely represent a single entity with variable morphology. Both are solitary, painless, well-circumscribed, and slowly growing lesions that often arise in the subcutis or dermis of the upper back, shoulder, neck or anterior head and neck regions of middle-aged to older men. They rarely exceed 5 cm in greatest dimension.

CYTOMORPHOLOGY OF SPINDLE CELL OR PLEOMORPHIC LIPOMA:

- fragments of mature adipose tissue
- myxoid background
- occasional multivacuolated lipoblast-like cells

Spindle Cell Lipoma:

- bland and uniform spindle cells
- ropy collagen fibers
- mast cells

Pleomorphic Lipoma:

- “floret” cells
- smudged chromatin

The spindle cell lipoma is characterized by a mixture of mature adipocytes and bland spindle cells in short fascicles. Hyaline, ropy collagen fibers and mast cells are typically seen.^{46,47} The pleomorphic lipoma shows predominantly mature adipocytes admixed with “pleomorphic” large, atypical stromal cells that have multiple hyperchromatic, floret-type nuclei (Fig. 16.4).^{24,48} Hybrids of spindle cell and pleomorphic lipoma are quite common.

DIFFERENTIAL DIAGNOSIS OF SPINDLE CELL OR PLEOMORPHIC LIPOMA:

- well-differentiated liposarcoma or atypical lipomatous tumor
- schwannoma
- myxoid liposarcoma
- myxofibrosarcoma, low grade
- solitary fibrous tumor
- dermatofibrosarcoma protuberans

A myxoid background and multivacuolated lipoblast-like cells are well-recognized diagnostic pitfalls associated with both variants, particularly the pleomorphic lipoma, and can lead to an erroneous diagnosis of a myxoid or well-differentiated liposarcoma or other myxoid or spindle cell neoplasms.^{44,48} This is particularly likely in cases that lack the classic floret cells or mature adipose tissue component. Spindle cell or pleomorphic lipoma is distinguished from liposarcoma by its typical anatomic distribution and superficial location, along with supportive immunohistochemistry (CD34 positivity in spindle cells) and cytogenetic findings (chromosomal aberrations of 13q and 16q, see Table 16.1). The positivity for CD34 and lack of S-100 protein in the spindle cells also helps to distinguish it from a schwannoma. Myxoid liposarcoma and myxofibrosarcoma have more prominent blood vessels and lack mature adipose tissue

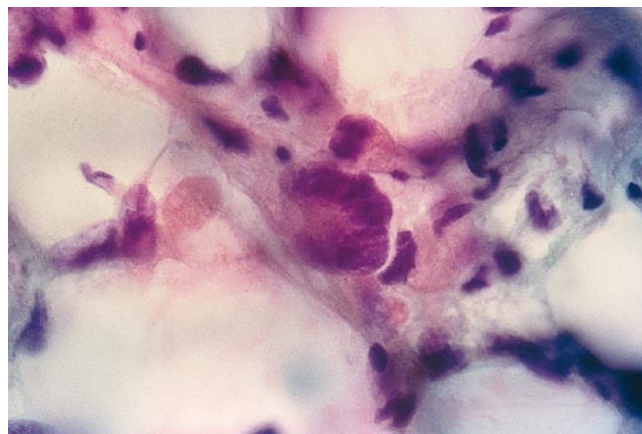


Figure 16.4 “Floret” cell. Multinucleated giant cells with nuclei in a wreath-like configuration are a characteristic but nonspecific finding in pleomorphic lipomas (Papanicolaou stain).

and collagen fibers. With a spindle-cell-predominant variant, the distinction from a dermatofibrosarcoma protuberans (DFSP) or solitary fibrous tumor, especially the fat-forming variant, can be difficult, because both mimics can have mature adipose tissue and CD34-positive spindle cells. Clinical correlation and cytogenetic findings are essential in difficult cases.^{24,47,49}

Well-Differentiated Liposarcoma or Atypical Lipomatous Tumor

Liposarcoma is the most common soft tissue sarcoma of adults, its incidence peaking in the fifth to seventh decades of life. It has three major forms: well-differentiated or dedifferentiated, myxoid or round cell, and pleomorphic. The terms *well-differentiated liposarcoma* and *atypical lipomatous tumor* are essentially synonymous. Well-differentiated liposarcoma applies to deep-seated lesions in the retroperitoneum, spermatic cord, and mediastinum, where recurrence and local aggressiveness are possible; atypical lipomatous tumor applies to lesions of the limbs and trunk that do not recur if adequately excised.⁴⁴

CYTOMORPHOLOGY OF WELL-DIFFERENTIATED LIPOSARCOMA OR ATYPICAL LIPOMATOUS TUMOR:

- lipid vacuoles of varying size
- atypical stromal cell nuclei
- lipoblasts

In general, tissue fragments of large but variably sized, univacuolated adipocytes (Fig. 16.5) are admixed with

blood and extruded lipid. Smaller multivacuolated cells with atypical, scalloped nuclei (lipoblasts) are present in some cases (Fig. 16.6). The presence of lipoblasts is not necessary for the diagnosis of liposarcomas, except for pleomorphic liposarcoma. The nuclei of the larger adipocytes range from small and compressed to large, round to oval, and hyperchromatic (Fig. 16.7). They are often multilobated or convoluted. Similar atypical nuclei are seen in fragments of collagenous stromal tissue.^{41,43,50}

DIFFERENTIAL DIAGNOSIS OF WELL-DIFFERENTIATED LIPOSARCOMA OR ATYPICAL LIPOMATOUS TUMOR:

- lipoma
- extruded lipid from normal fat
- fat necrosis (see discussion on lipoma and Fig. 16.2)
- spindle cell or pleomorphic lipoma

Extensive sampling of a large liposarcoma is necessary because diagnostic areas can be extremely focal.^{11,24,43,48,51} The first impression might be that of a lipoma, but the clinical presentations of lipomas and liposarcomas are generally so different that this rarely presents a significant dilemma. Nevertheless, careful examination of the specimen is important to identify the sometimes subtle nuclear and cellular alterations of a well-differentiated liposarcoma (see Fig. 16.5). Benign lipid droplets entrapped in blood are a common finding whenever any fatty tissue, including normal adipose tissue, is sampled. Its non-specific nature is revealed by the absence of nuclei

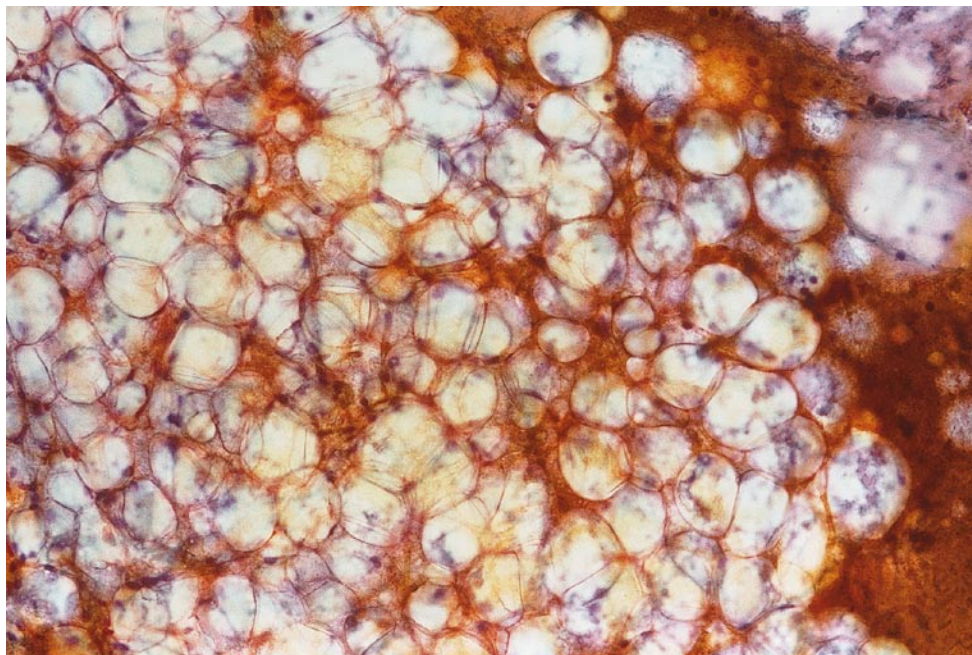


Figure 16.5 Well-differentiated liposarcoma. The much-touted variation in adipocyte size can be subtle and easily overlooked (compare with Fig. 16.1), (Papanicolaou stain).

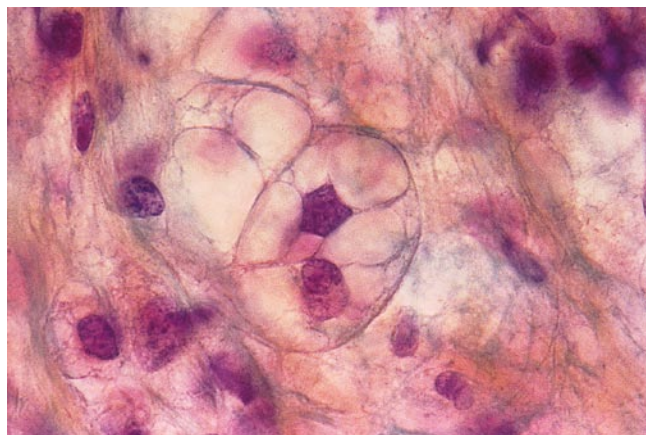


Figure 16.6 Lipoblast. The hyperchromatic nuclei of lipoblasts are scalloped, resulting from multiple impinging cytoplasmic lipid droplets (Papanicolaou stain).

(Fig. 16.8). Fat necrosis is comprised of lipophages (see Fig. 16.2), whose cytoplasm is foamy and filled with small vacuoles, whereas the cytoplasm of adipocytes and lipoblasts is optically clear. Some of the multilobated hyperchromatic cells of a liposarcoma can resemble the floret cells of a pleomorphic lipoma; clinical and radiographic correlation is the key to this distinction. Giant marker and ring chromosomes derived from 12q13-15 are characteristic of well-differentiated liposarcoma (see Table 16.1) and help to differentiate it from its benign mimics like hibernoma and spindle cell or pleomorphic lipoma. The latter have their own characteristic cytogenetic alterations. With lipomatous tumors, one rarely gets enough material from an FNA for a conventional karyotype. They are more amenable to molecular diagnostic or FISH analysis.

A rare variant of well-differentiated liposarcoma, the *inflammatory liposarcoma*, yields moderately cellular smears with abundant small lymphocytes, larger lymphoid cells, and plasma cells. The scattered large, atypical cells with multiple or multilobated nuclei of this variant evoke the unusual differential diagnosis of Hodgkin lymphoma, anaplastic large cell lymphoma, inflammatory myofibroblastic tumor, and metastatic carcinoma.⁵²

Pleomorphic Liposarcoma

Pleomorphic liposarcoma is a rare malignancy, accounting for about 5% of liposarcomas. It has a striking predilection for the limbs and a bleak prognosis. It is distinguished from other high-grade pleomorphic sarcomas (the malignant fibrous histiocytoma-like family) by the presence of multivacuolated highly atypical lipoblasts.

CYTOMORPHOLOGY OF PLEOMORPHIC LIPOSARCOMA:

- hypercellular smear
- pleomorphic cells with marked nuclear atypia
- variable number of atypical lipoblasts
- mitoses and necrosis

Smears are moderately to highly cellular. The malignant cells are dyshesive and markedly anaplastic and pleomorphic. They range in contour from polygonal to spindle-shaped. Nuclei are bizarre and occasionally scalloped, with coarse chromatin and prominent nucleoli. The abundant cytoplasm varies from homogeneous to multivacuolated, with vacuoles of varying sizes. Mitoses

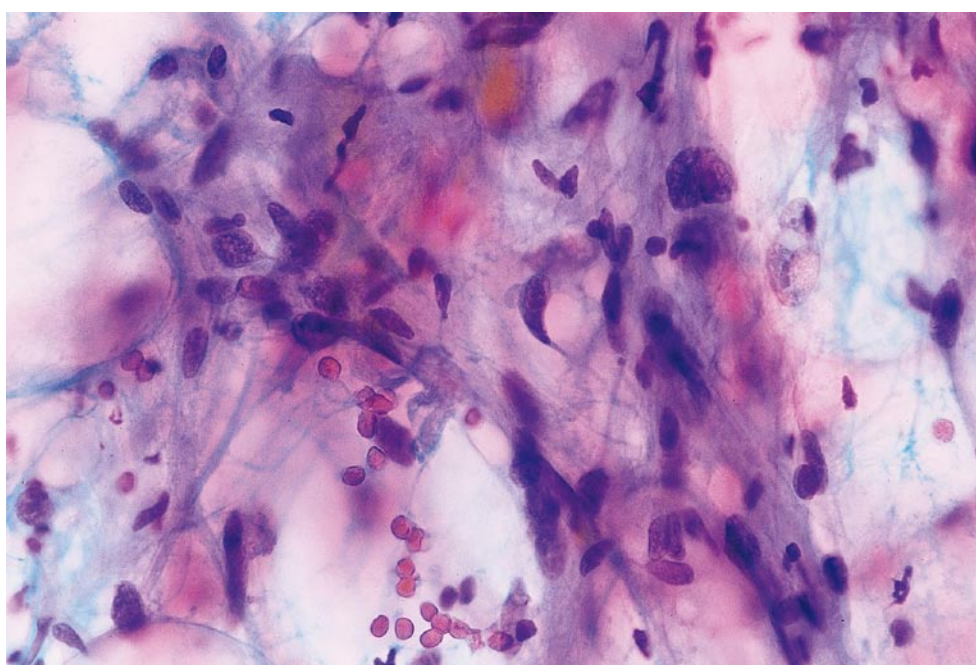


Figure 16.7 Well-differentiated liposarcoma. Atypical adipocyte nuclei in deep-seated lesions denote malignancy even when classic lipoblasts are not readily identified (Papanicolaou stain).

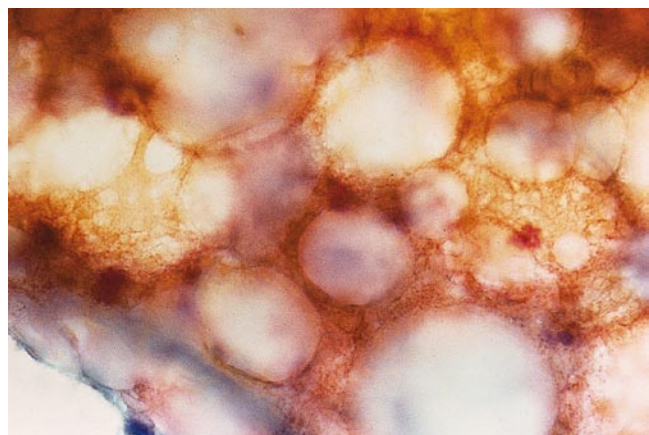


Figure 16.8 Extruded lipid. A high clinical suspicion for liposarcoma might lead to an overinterpretation of extruded lipid from normal fat at the time of rapid evaluation (Papanicolaou stain).

and necrosis are usually present. Extensive sampling to identify unequivocal atypical lipoblasts is necessary to separate pleomorphic liposarcomas from pleomorphic sarcoma of another lineage. Cytogenetically, pleomorphic liposarcoma exhibits increased chromosome numbers with complex rearrangements, a profile, unfortunately, of no diagnostic value.^{24,53,54}

MYXOID NEOPLASMS

The diverse and mostly unrelated myxoid soft tissue lesions share one (often striking) morphologic feature: abundant, homogeneous, myxoid matrix. In most instances, it appears as an amorphous, granular film that stains pale blue-green with the Papanicolaou stain (Fig. 16.9) and a striking blue-violet with Romanowsky stains.

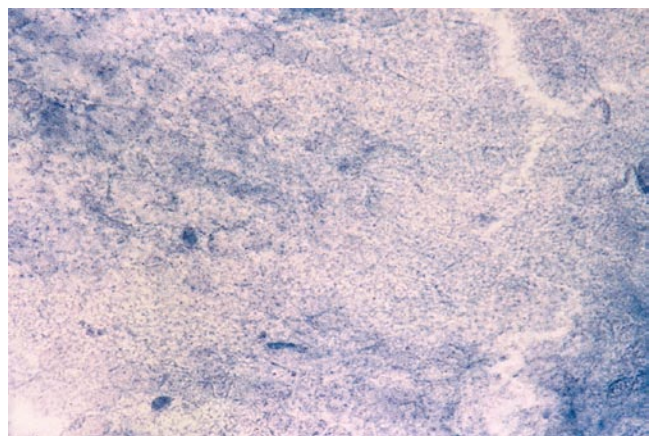


Figure 16.9 Myxoid matrix (ganglion cyst). The copious matrix material of a ganglion cyst has a granular appearance common to most myxoid soft tissue lesions (Papanicolaou stain).

A myxoid picture is produced by a broad spectrum of lesions that includes reactive conditions like nodular fasciitis, benign myxoid tumors, and myxoid sarcomas. In benign lesions, the ground substance is fine and semitranslucent. It is denser and almost fibrillar in some malignant lesions. Additionally, benign lesions generally have lower cellularity and less nuclear atypia than malignant neoplasms, but exceptions do occur. Limited sampling may yield hypocellular smears with abundant matrix and result in an erroneous low-grade assignment. Despite the challenges, myxoid lesions, particularly the low-grade ones, can often be subtyped based on cytomorphology alone with accuracy rates up to 81%.⁶⁰ Cytogenetic analysis is helpful in resolving the differential diagnosis of some of the malignant lesions, like myxoid liposarcoma, low-grade fibromyxoid sarcoma, and extraskeletal myxoid chondrosarcoma (see Table 16.1).

Intramuscular Myxoma

Intramuscular myxoma is a benign, painless, slowly growing, well-circumscribed but unencapsulated neoplasm of adults aged 30 to 70 years, usually involving the muscles of the thigh or lower limb girdle. A *cellular myxoma* is a morphologic variant that has increased cellularity and a vascular component, but it lacks the nuclear atypia and hyperchromasia of low-grade myxofibrosarcoma. It has a slight risk of local recurrence.⁵⁵ The *juxta-articular myxoma* is cytologically similar to a cellular myxoma, but it occurs adjacent to large joints, especially the knee.

CYTOMORPHOLOGY OF INTRAMUSCULAR MYXOMA:

- hypocellular
- a film of granular matrix material
- absent or scant vascular component
- uniform cells with a bland nucleus
- long, fibrillary cytoplasmic processes
- multinucleated atrophic muscle fibers

FNAs from intramuscular myxomas are hypocellular, with abundant, granular myxoid matrix. The vascular component consists of rare small vessels. Rare fibroblast-like cells, isolated and in loose clusters, are uniform in size and display a variety of shapes: round to oval, spindle-shaped, stellate, and polygonal (Fig. 16.10). Their small, round to oval nuclei lack atypia and have fine, uniform chromatin with small nucleoli. The cytoplasm is finely granular with long, fibrillary cytoplasmic processes and occasional small vacuoles. Macrophages and mast cells can be present. Often, multinucleated skeletal muscle fibers, sometimes atrophic, are strewn about. Mitoses are virtually never observed.⁵⁶⁻⁵⁸



Figure 16.10 Intramuscular myxoma. Loose aggregates of fibroblast-like cells appear to swim through a sea of myxoid ground substance (Papanicolaou stain).

DIFFERENTIAL DIAGNOSIS OF INTRAMUSCULAR MYXOMA:

- ganglion cyst
- nodular fasciitis
- low-grade myxofibrosarcoma
- low-grade fibromyxoid sarcoma
- myxoid or round cell liposarcoma
- extraskeletal myxoid chondrosarcoma

Aspirates from ganglion cysts contain only abundant granular myxoid material and occasional histiocytes; the spindle-shaped, stellate, or polygonal myxoma cells are absent. Nodular fasciitis shows a more haphazard cellular arrangement of polymorphic myofibroblasts and ganglion-like cells, often with interspersed inflammatory cells. Both myxofibrosarcoma and myxoid or round cell liposarcoma have a more prominent vascular component than the typical intramuscular myxoma. Although low-grade myxofibrosarcoma and low-grade fibromyxoid sarcoma have notable nuclear atypia, their distinction from cellular myxoma can be difficult. Adequate sampling from different parts of the mass is essential to avoid missing a low-grade fibromyxoid sarcoma, which has alternating areas of myxoid and collagenous stroma. The stroma in extraskeletal myxoid chondrosarcoma is more brightly magenta and fibrillar rather than blue-purple and granular as seen in a myxoma, and the lace-like arrangement of tumor cells is more uniform than that of myxoma cells (Fig. 16.17).

Myxofibrosarcoma, Low and High Grade (Myxoid Malignant Fibrous Histiocytoma)

Myxofibrosarcoma, also known as myxoid malignant fibrous histiocytoma (myxoid MFH), is one of the most

common sarcomas. It typically occurs in the extremities of elderly patients, principally during the sixth to eighth decades of life. Usually dermal or subcutaneous, it involves deeper soft tissues in only one third of cases. Up to 60% of cases recur locally regardless of grade, and they tend to become progressively higher grade with each recurrence. Only higher-grade lesions have the potential to metastasize to lungs, bones, and lymph nodes.

CYTOMORPHOLOGY OF MYXOFIBROSARCOMA:

- variable amounts of myxoid matrix
- short segments of curved, collagenized vessels
- mildly atypical spindle and stellate cells (low-grade tumors)
- marked nuclear pleomorphism, multinucleation, and necrosis (high-grade tumors)
- pseudolipoblasts

Myxofibrosarcomas are morphologically quite variable, ranging from low-grade to high-grade tumors. In general, the amount of myxoid material is inversely proportional to cellularity and grade. The curvilinear vessels that are characteristic of these tumors in histologic sections are absent from smears in many cases, but when present, they appear as short segments of collagenized capillaries best seen in paucicellular areas (Fig. 16.11). The neoplastic cells are randomly distributed and dyshesive. Occasional small aggregates occur, and higher-grade lesions contain large cell groups and tissue fragments. The majority of cells in a low-grade tumor are spindle-shaped, with occasional tadpole, stellate, and polygonal forms. Nuclear pleomorphism and atypia increase with grade (Fig. 16.12A). In higher-grade lesions, binucleation and multinucleation are more prominent. Irrespective of grade, tumor cells with vacuolated cytoplasm, resembling lipoblasts but containing acid mucins rather than lipid, are found (Fig. 16.12B).^{9,56,59-61} Mitotic activity is rare. Necrotic cellular debris is disseminated in the background.

DIFFERENTIAL DIAGNOSIS OF MYXOFIBROSARCOMA:

- cellular myxoma
- nodular fasciitis
- myxoid liposarcoma
- low-grade fibromyxoid sarcoma

Cellular myxoma and nodular fasciitis lack the curvilinear vessels and nuclear atypia of myxofibrosarcoma. Myxoid liposarcoma contains delicate branching vessels and true lipoblasts and usually does not have much nuclear atypia or pleomorphism. The distinction between low-grade myxofibrosarcoma and the similarly

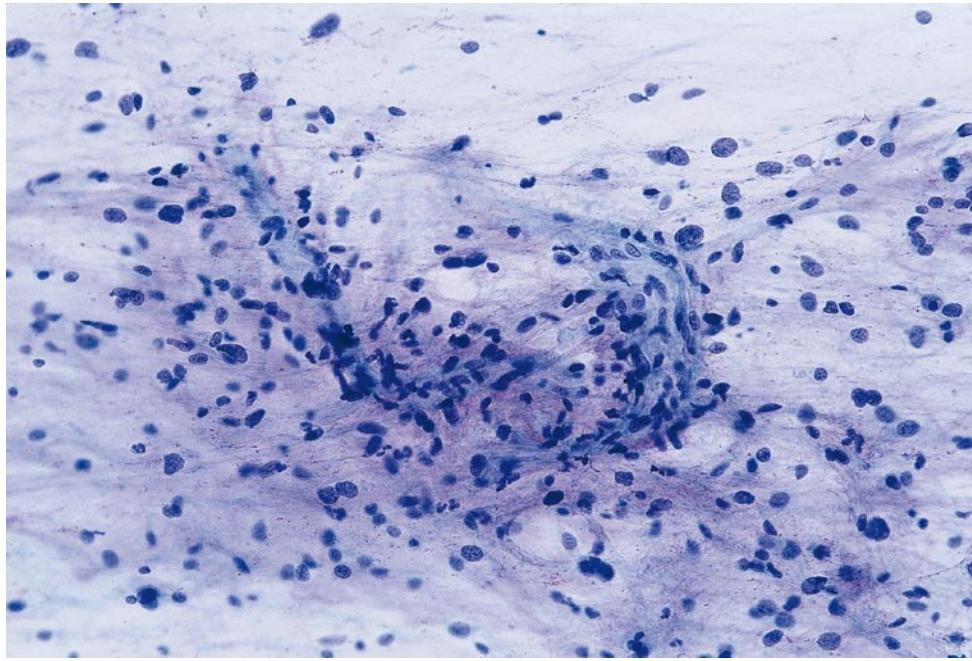
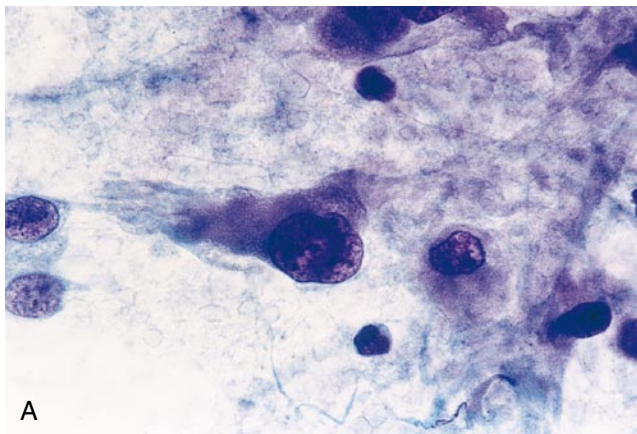
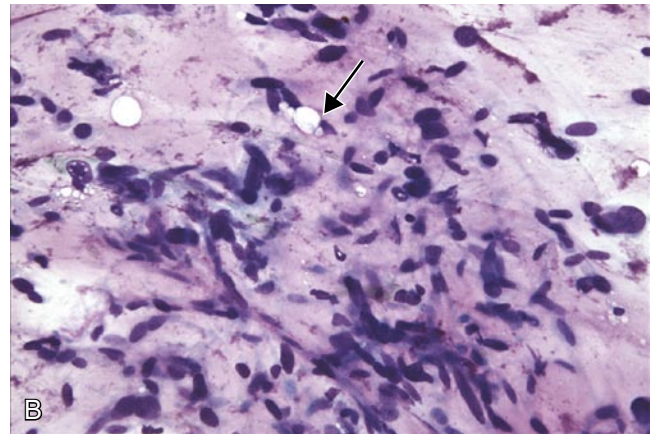


Figure 16.11 Myxofibrosarcoma. The vascular component, when present, manifests itself as short segments of thick-walled vessels with adherent matrix and tumor cells (Papanicolaou stain).



A



B

Figure 16.12 Myxofibrosarcoma. A, Numerous pleomorphic fibroblast-like cells and giant cells in a myxoid background should suggest the diagnosis of myxofibrosarcoma (Papanicolaou stain). B, Pseudolipoblasts (arrow) contain acid mucins rather than lipid (Romanowsky stain).

named low-grade fibromyxoid sarcoma is difficult but possible, especially in conjunction with clinical and cytogenetic findings.

Low-Grade Fibromyxoid Sarcoma

Low-grade fibromyxoid sarcoma, first described by Evans, is clinically, morphologically, and cytogenetically different from a low-grade myxofibrosarcoma.⁶² It affects mainly younger adults in the third to fifth decades and arises in the deep soft tissue of the thigh, inguinal region, shoulder, and perineum. Despite its deceptively bland morphology, at least 30% of cases eventually metastasize after a long indolent course.⁴⁴

CYTOMORPHOLOGY OF LOW-GRADE FIBROMYXOID SARCOMA:

- abundant myxoid matrix material
- uniform fibroblast-like spindle cells with mild nuclear atypia
- no significant vascularity or nuclear pleomorphism

Histologically, low-grade fibromyxoid sarcoma has a characteristic whorled arrangement of bland spindle cells with alternating areas of myxoid and collagenous stroma, but the cytologic findings, described in only a few case reports,⁶³⁻⁶⁵ are somewhat non-specific. Often the myxoid, hypocellular areas are sampled, yielding a homogeneous population of fibroblast-like spindle cells

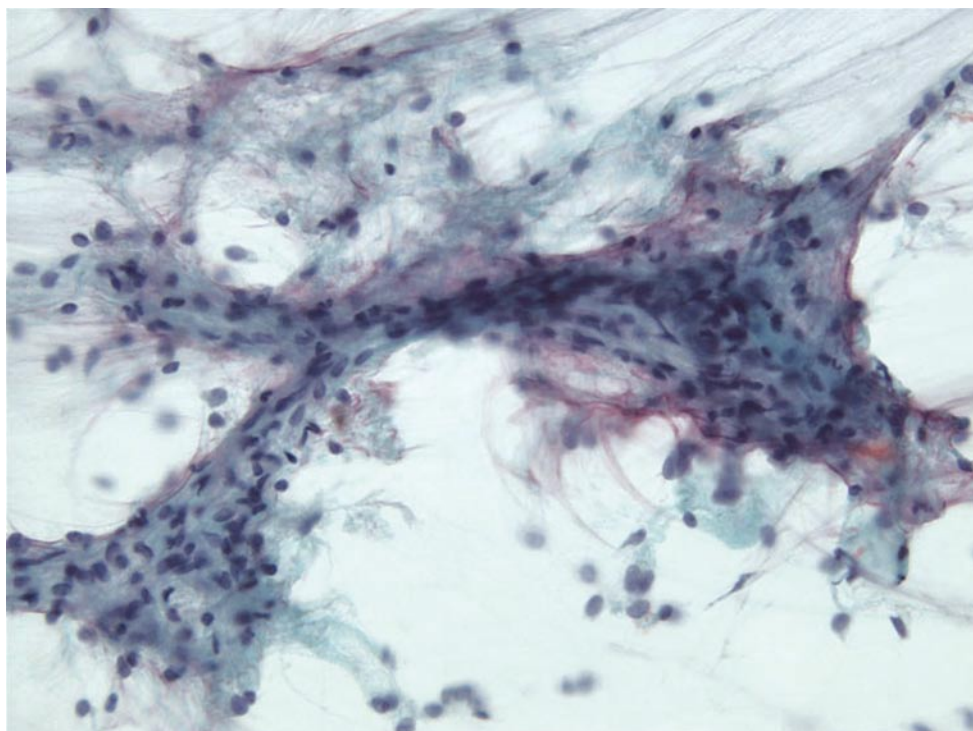


Figure 16.13 Low-grade fibro myxoid sarcoma. Uniformly bland fibroblasts are embedded in a myxoid matrix (Papanicolaou stain).

with slightly plump but bland nuclei in a myxoid background. Vascularity is variable depending on the area sampled (Fig. 16.13).

DIFFERENTIAL DIAGNOSIS OF LOW-GRADE FIBROMYXOID SARCOMA:

- cellular myxoma
- low-grade myxofibrosarcoma
- myxoid variant of smooth muscle or neuroectodermal tumors

A cellular myxoma is usually less cellular and poorly vascularized. Low-grade myxofibrosarcoma has more notable nuclear atypia, and, if present, the curvilinear vessels of that tumor are helpful. Clinically, low-grade myxofibrosarcoma tends to occur in the superficial soft tissues of elderly patients, whereas low-grade fibromyxoid sarcoma is usually situated in the deep soft tissues of young adults. Immunohistochemical studies can be helpful in distinguishing it from smooth muscle or neuroectodermal neoplasms with myxoid features: Vimentin is usually the only positive marker in most cases of low-grade fibromyxoid sarcoma. A chromosomal abnormality $t(7;16)(q34;p11)$ typical of low-grade fibromyxoid sarcoma can also be a reliable diagnostic aid (see Table 16.1).^{66,67}

Myxoid or Round Cell Liposarcoma

Myxoid liposarcoma and round cell liposarcoma represent two ends of the morphologic spectrum of a single

entity. They share the same reciprocal chromosomal translocations (see Table 16.1). Pure myxoid liposarcomas form the low-grade end of the spectrum; an increase in the round cell or hypercellular component culminates in the high-grade, pure round cell liposarcoma. They occur in a slightly younger population than the other liposarcomas, primarily in the third to fifth decades, and have a predilection for the deep soft tissue of the lower limbs, especially the thigh.

CYTOMORPHOLOGY OF MYXOID OR ROUND CELL LIPOSARCOMA:

- abundant granular myxoid matrix
- delicate, branching, thin-walled capillaries
- spindle-shaped to oval cells with occasional small cytoplasmic vacuoles
- univacuolated and bivacuolated lipoblasts ("signet-ring cell"-like lipoblasts), especially along capillaries
- increased number of dispersed round cells with scant cytoplasm and prominent nucleoli (round cell liposarcoma)

Smears are moderately to markedly cellular. Regardless of grade, myxoid or round cell liposarcomas have abundant granular myxoid matrix with arborizing, thin-walled capillaries of uniform caliber (Fig. 16.14A). Lower-grade myxoid liposarcomas contain tissue fragments with a prominent myxoid stroma, numerous optically clear lipoblasts of varying sizes, and smaller cells

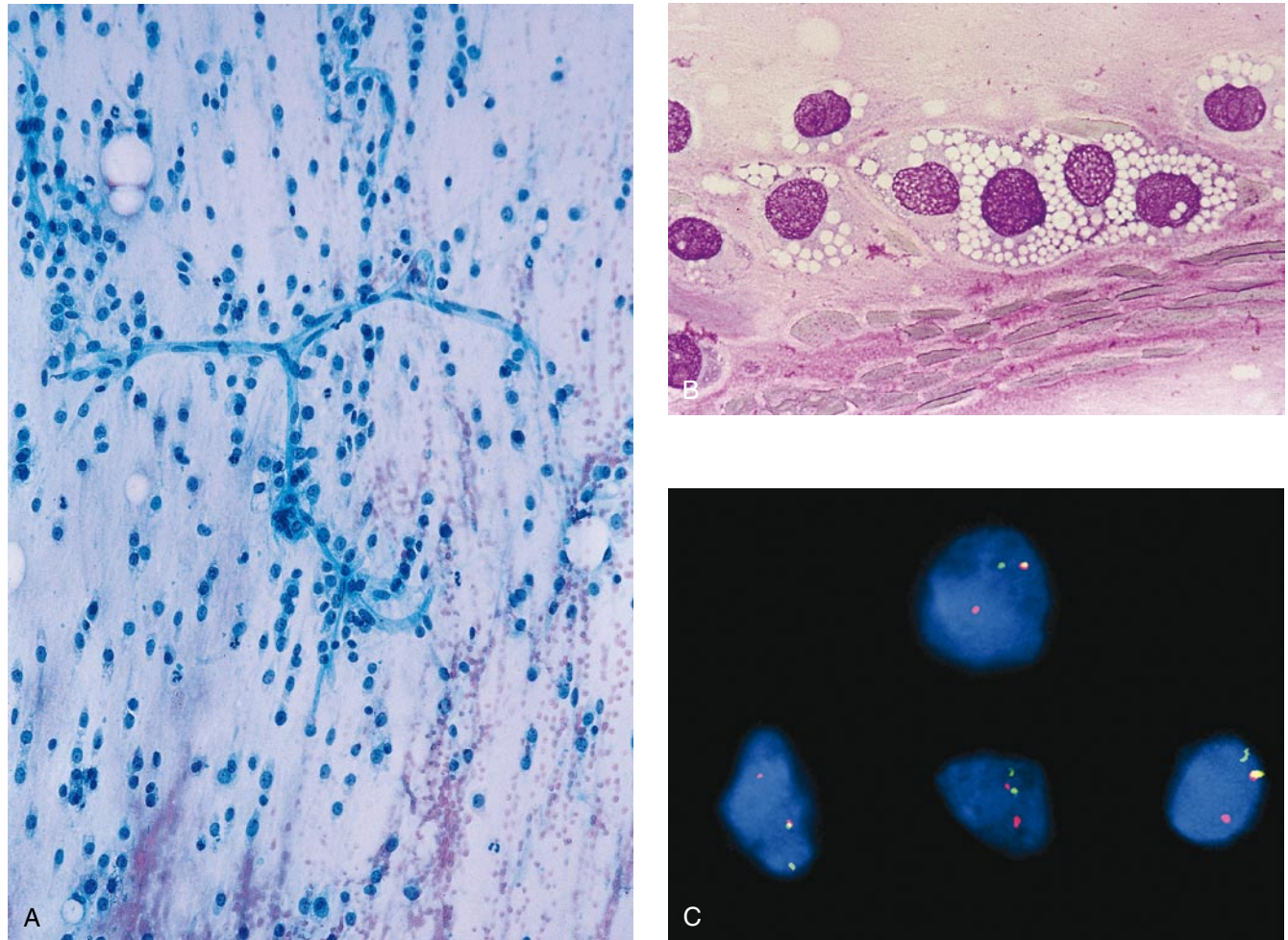


Figure 16.14 Myxoid or round cell liposarcoma. A, Delicate “chicken-wire” capillaries are easily obtained by aspiration and are seen in abundance on smears (Papanicolaou stain). B, Some cells feature numerous small cytoplasmic vacuoles best visualized with air-dried preparations (Romanowsky stain). C, Fluorescence in-situ hybridization (FISH) is often performed with centromeric (red) and telomeric (green) probes that flank the *CHOP* (*DDIT3*) gene breakpoint. There is one normal chromosome (probes adjacent = yellow), but the split-apart green and red signals indicate a translocation on the other chromosome in the four tumor cells in this field. (Courtesy of Dr. Paola Dal Cin, Brigham and Women’s Hospital, Boston).

with scant cytoplasm and indistinct cell borders. The lipoblasts are usually small, univacuolated or bivacuolated, resembling signet-ring cells (“signet-ring cell”-like lipoblasts), and are often present along the capillaries. The small cells of a lower-grade myxoid liposarcoma are uniformly oval to fusiform (Fig. 16.14B). Their monomorphic nuclei are oval, with evenly distributed, finely granular chromatin and up to two inconspicuous nucleoli.

In contrast, higher-grade tumors have a greater component of isolated and loosely clustered cells.^{11,22,24,43,56,60,61} These are substantially larger and rounder, with more anisokaryosis and hyperchromasia of the centrally placed nuclei and multiple, prominent nucleoli. The cytoplasm is scant, more densely staining, but rarely vacuolated. Resulting from its prognostic significance, the degree of round cell or hypercellular component should be documented. The detection of the chromosomal abnor-

malities $t(12;16)(q13;p11)$ or $t(12;22)(q13;q12)$ typical of myxoid or round cell liposarcoma by either karyotype or FISH (Fig. 16.14C) can help distinguish it from other myxoid tumors (see Table 16.1).^{11,22,24,43,56,60}

Myxofibrosarcoma-Like Dedifferentiated Liposarcoma

Dedifferentiation, a concept first introduced by Evans in 1979,^{67a} refers to “tumors containing distinct areas of well-differentiated liposarcoma and cellular nonlipogenic spindle cell or pleomorphic sarcoma.” Dedifferentiation is not limited to liposarcomas.

Dedifferentiated liposarcoma has a variable morphologic picture, but most cases resemble either a pleomorphic undifferentiated (“malignant fibrous

histiocytoma"-like) sarcoma or a myxofibrosarcoma. When the typical morphology of a myxofibrosarcoma is encountered in the retroperitoneum, the diagnosis of a dedifferentiated liposarcoma should be given serious consideration.^{68,69}

**CYTOMORPHOLOGY OF
MYXOFIBROSARCOMA-LIKE
DEDIFFERENTIATED LIPOSARCOMA:**

- abundant granular myxoid matrix
- thick-walled branching vessels
- spindle cells with mild to moderate nuclear atypia
- occasional multinucleated tumor cells

Smears are moderately to highly cellular with a prominent granular, myxoid background. Dispersed spindled to stellate cells surround loosely cohesive tumor cell clusters arrayed around long vascular channels. The vessels show complex branching similar to the delicate capillaries of myxoid or round cell liposarcoma, but their walls are notably thicker (Fig. 16.15). Most individual cells have round to oval, hyperchromatic nuclei with evenly distributed chromatin and indistinct nucleoli. The cytoplasm is scant to moderate and occasionally vacuolated. Other cells have more abundant, stellate-shaped cytoplasm, slightly larger and irregular nuclei with smudgy chromatin, and a greater tendency toward binucleation or multinucleation. Rare diagnostic lipoblasts may be seen.

Cytogenetic analysis and clinical details are important for the correct diagnosis.

Lipoblastoma

Lipoblastoma is a rare, benign tumor affecting mainly males aged 3 years or younger. It involves the superficial subcutaneous tissues of the limbs, especially the lower extremities, as a slowly growing circumscribed mass. More deeply seated lesions tend toward larger size and a diffusely infiltrative growth pattern (lipoblastomatosis). Local recurrence is uncommon and is primarily restricted to the infiltrative lesions.

CYTOMORPHOLOGY OF LIPOBLASTOMA:

- myxoid matrix with lipid vacuoles
- prominent thin, branching capillaries
- three types of cells: bland lipoblasts, primitive spindle cells, and mature adipocytes

Smears are moderately cellular, with cohesive clusters and sheets of bland lipoblasts and primitive spindle-shaped cells embedded in a myxoid background with fatty vacuoles. Myxoid material can be abundant or sparse and restricted to small-sized to medium-sized tissue fragments with irregular borders. Delicate branching capillaries are conspicuous. Bare nuclei can be widespread, and variable amounts of mature adipocytes are seen depending on the degree of maturation.

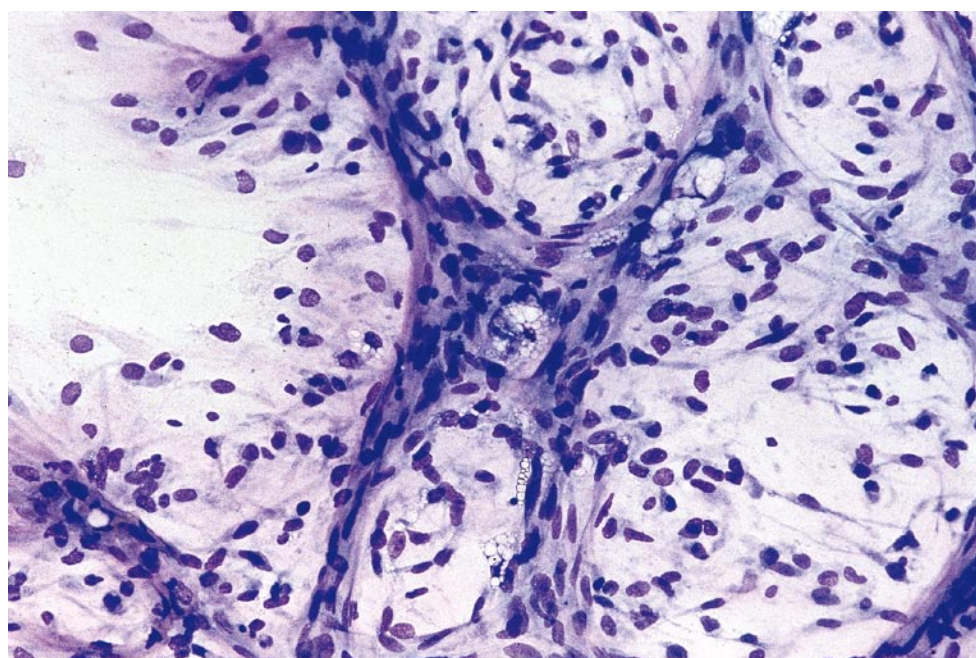


Figure 16.15 Myxofibrosarcoma-like dedifferentiated liposarcoma. Noticing the complex, branching, thick-walled vasculature of a dedifferentiated liposarcoma can aid in its distinction from other myxoid lesions (Romanowsky stain).

The lipoblasts are generally small, round, and uniform in appearance. Chromatin is delicate or moderately coarse, and nucleoli are largely absent.^{43,70,71} Nuclear atypia is limited to focal minimal nuclear membrane irregularities; it is not as prominent as seen with the lipoblasts of a liposarcoma.

In addition to affecting a younger-aged population, lipoblastoma is distinguished from its primary diagnostic mimic, myxoid liposarcoma, by the absence of nuclear pleomorphism, less abundant myxoid stroma, and the presence of solid sheets of tumor cells. Cytogenetic analysis is helpful in their distinction in difficult cases (see [Table 16.1](#)).^{43,70,71}

Chordoma

Chordoma is the only malignant neoplasm derived from notochordal elements. This rare, midline lesion usually occurs at one of the two ends of the axial skeleton. Patients can have symptoms for months to years before diagnosis. Chordoma has a relatively indolent, protracted course, often with multiple recurrences. Metastases occur in a minority of cases.

CYTOMORPHOLOGY OF CHORDOMA:

- granular and fibrillary myxoid matrix
- cohesive clusters and cords of neoplastic cells
- comparatively large cells
- physaliphorous cells

Cohesive clusters and cords of round to cuboidal cells are found in a rich myxoid matrix. The cells are larger than those of other myxoid neoplasms. Mild nuclear pleomorphism is present, with more prominent nucleoli in the more pleomorphic cells. The cytoplasm contains conspicuous cytoplasmic vacuoles ([Fig. 16.16](#)). Some cells have an abundance of vacuolated cytoplasm and are called “physaliphorous” (from the Greek “physallis,” meaning bubble); the edges of the vacuoles in physaliphorous cells resemble thin spider legs extending from the nucleus to the cell borders. Univacuolated signet ring-like cells and nonvacuolated smaller cuboidal cells with moderate amounts of granular cytoplasm are also present.

DIFFERENTIAL DIAGNOSIS OF CHORDOMA:

- chondrosarcoma, myxoid
- myxopapillary ependymoma
- metastatic mucinous adenocarcinoma

The cytologic differential diagnosis includes chondrosarcoma, mucinous adenocarcinoma, and myxopapillary ependymoma. In difficult cases, immunohistochemical studies are of help. Both chordomas and adenocarcinomas are positive for cytokeratins; but chordomas are also positive for S-100 protein. Chondrosarcomas and ependymomas are negative for cytokeratins, but chondrosarcomas are positive for S-100, and ependymomas express glial fibrillary acid protein (GFAP).^{22,61,72-74}

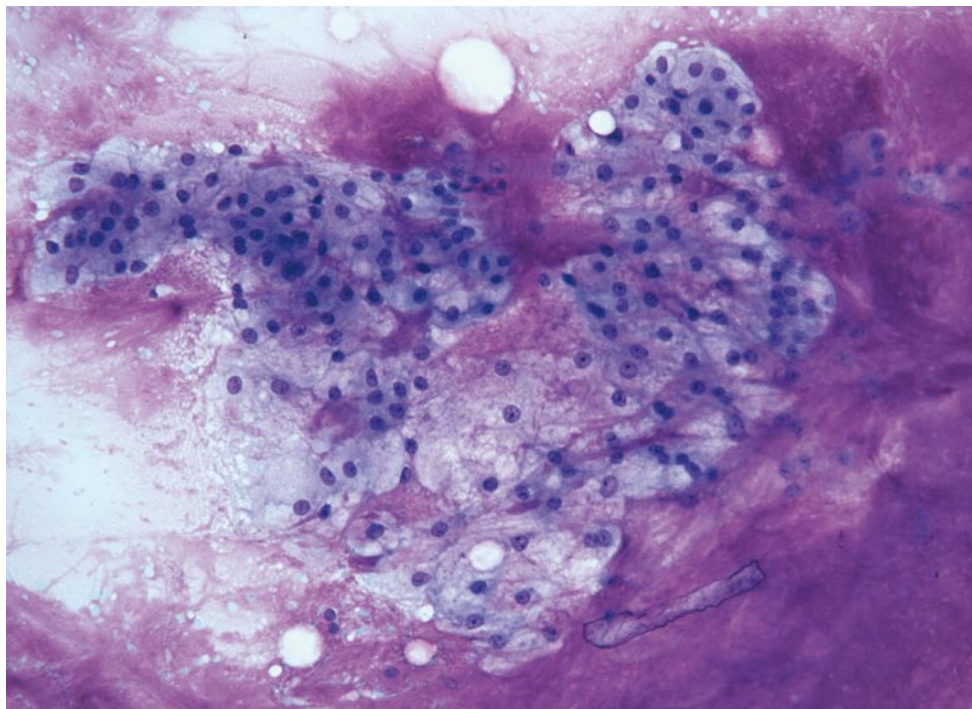


Figure 16.16 Chordoma. The cells are much more cohesive and epithelioid than the cells of other myxoid sarcomas (Romanowsky stain).

Extraskelatal Myxoid Chondrosarcoma

Extraskelatal myxoid chondrosarcoma (EMC) is a slowly growing, painless tumor of adults over 35 years of age. It involves the deep soft tissues of the proximal limbs, especially the thigh and popliteal fossa. Traditionally regarded a low-grade indolent malignancy with only late local recurrences, 40% to 50% of cases are now acknowledged to metastasize after 10 or more years.

CYTOMORPHOLOGY OF EXTRASKELETAL MYXOID CHONDROSARCOMA:

- consistent appearance from case to case
- bright magenta fibrillary stroma in Romanowsky-stained preparations
- variable degree of lacunar formation
- cords and lace-like arrangements
- uniform, fusiform, bland tumor cells

Of all the myxoid soft tissue neoplasms, EMC shows the least morphologic variation from one tumor to another. The myxoid matrix has a fibrillary texture that is distinct from the granular appearance of most of the other myxoid lesions. It stains bright magenta in air-dried preparations (Fig. 16.17A). Among the myxoid sarcomas, EMC has the fewest vascular structures. Chondroblast-like lacunar formation can occur, but no definite hyaline cartilage differentiation has been described.^{22,56,60,75-77}

Smears are moderately cellular, with plump spindle-shaped or oval tumor cells arranged in a lacelike pattern of anastomosing, loosely cohesive cords and nests. The malignant cells are uniform and lack nuclear pleomorphism (Fig. 16.17B). Nuclei are round or oval and hyperchromatic with finely stippled chromatin. The single nucleolus is small and inconspicuous. Nuclear grooves

and clefts are common. Cytoplasm is homogeneous, scant to moderately abundant, and often appears wispy and tapered, with well-defined cell borders.^{22,56,60,75-78}

There is no specific immunoprofile for EMC; less than 20% of cases show immunoreactivity for S-100 protein. A characteristic translocation t(9;22)(q22;q12), which involves the *EWS* gene, is a consistent cytogenetic finding and can be of diagnostic value (see Table 16.1 and Fig. 16.28).^{44,76}

SPINDLE CELL NEOPLASMS

The benign tumors in this group yield intact tissue fragments with few or no dispersed cells, whereas the malignant lesions feature more cell dissociation, greater cellularity, necrosis, and mitotic activity. One important distinction is determining whether the mass is of smooth muscle, nerve sheath, myofibroblastic, or other mesenchymal origin. Although some characteristic cytomorphologic features can suggest a specific entity or line of differentiation, a definitive distinction depends on immunohistochemical studies using a panel of antibodies (Table 16.2).^{9,14,16,42} Spindle cell carcinoma and melanoma often need to be considered in the differential diagnosis as well.

Leiomyosarcoma

Leiomyosarcomas are subdivided into four clinical subtypes: intra-abdominal, subcutaneous or deep soft tissue, cutaneous, and vascular. Intra-abdominal tumors (40% to 45%) occur in older females in the retroperitoneum, mesentery, or omentum. Metastases to lung and liver are common. Subcutaneous and deep soft tissue tumors (20% to 30%) involve the extremities, particularly the thigh, with a slight male predominance. Cutaneous tumors (15% to 20%) affect younger adults, also with a male predominance. They are often painful lesions of

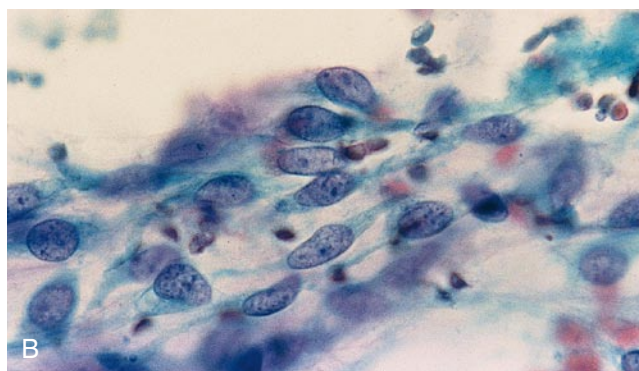
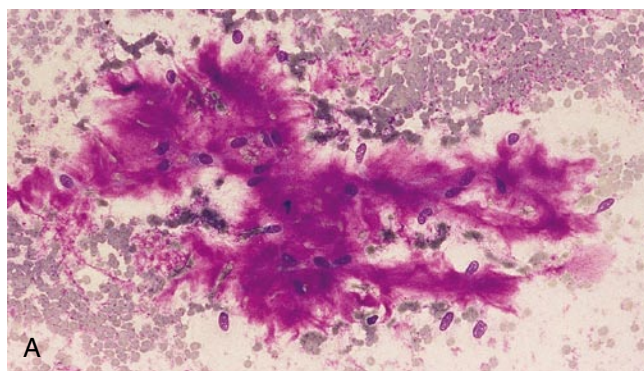


Figure 16.17 Extraskelatal myxoid chondrosarcoma. A, The extraskelatal myxoid chondrosarcoma has a distinctive fibrillar chondromyxoid matrix seen only to a lesser degree in some chordomas (Romanowsky stain). B, Long cytoplasmic extensions link one cell to another, often conferring a lace-like appearance (Papanicolaou stain).

TABLE 16.2 IMMUNOPROFILE OF SPINDLE CELL NEOPLASMS

Tumor	SMA/Desmin	S-100 Protein	CD34	c-kit	CK/EMA	β-catenin (nuclear)
Spindle cell lipoma	–	+ adipocytes	+ spindle cells	–	–	–
Schwannoma	–	+ diffuse	–	–	–	–
MPNST	–	+ focal	–/+	–	–/+	–
Synovial sarcoma*	–	–/+	–	–	+	–/+
GIST	–/+	–	+	+	–	–
Leiomyoma/ leiomyosarcoma	+ diffuse	–	–	–	–/+	–
SFT*	–	–	+	–	–	–/+
Fibromatosis	–	–	–	–	–	+

*D99 and bcl-2 are also positive.

GIST, gastrointestinal stromal tumors; MPNST, malignant peripheral nerve sheath tumor; SFT, solitary fibrous tumor.

the limbs and typically recur locally. Because it is now widely believed that a cutaneous leiomyosarcoma never metastasizes, the term *atypical intradermal smooth muscle neoplasm* is favored over *cutaneous leiomyosarcoma*.⁴⁴ Finally, vascular leiomyosarcomas (5%) occur adjacent to muscular-walled blood vessels, particularly the inferior vena cava and large veins of the lower limb in older adults. Leiomyosarcomas are immunoreactive for smooth muscle actin (SMA), desmin, and caldesmon. They are negative for S-100 protein. About one third of cases exhibit positivity for cytokeratins and epithelial membrane antigen (EMA).

CYTOMORPHOLOGY OF LEIOMYOSARCOMA:

- fascicular tissue fragments
- spindle-shaped cells
- “cigar-shaped” nuclei, some with indentation
- naked nuclei
- abundant homogeneous, finely granular cytoplasm
- mitoses

The cellular arrangement is variable; loosely cohesive clusters, closely packed tissue fragments with rigid edges (Fig. 16.18A), and short fascicles with elongated cells are seen. The percentage of isolated cells increases with tumor grade. In the *myxoid variant*, occasional hyaline stromal fragments (with blood vessels) contain a diffuse, granular ground substance. A parallel, side-by-side arrangement of tumor cells is common. The centrally placed nucleus is generally oval to elongated, with blunted or rounded ends (“cigar-shaped”; Fig. 16.18B) that are often indented. Naked nuclei are common. Anisokaryosis, pleomorphism, and multinucleation increase with tumor grade and are prominent features of the *pleomorphic variant*. The cytoplasm is generally abundant, bipolar, and homogeneously granular. Mitoses are common, and atypical forms are seen

in higher-grade lesions. The presence of even a single mitosis in a deep-seated smooth muscle tumor is highly suspicious for malignancy. Necrosis can be prominent in poorly differentiated tumors.^{9,14,42,79,80}

DIFFERENTIAL DIAGNOSIS OF LEIOMYOSARCOMA:

- leiomyoma
- schwannoma
- malignant peripheral nerve sheath tumor
- gastrointestinal stromal tumor (GIST)
- solitary fibrous tumor
- desmoid fibromatosis

Most leiomyosarcomas can be distinguished from a leiomyoma because the former has more generalized nuclear atypia, more mitotic activity, and necrosis. Unless attached to the uterus or the gastrointestinal (GI) tract, any deep-seated smooth muscle tumor should be considered malignant until proven otherwise. Nerve sheath tumors have wavy, “fishhook” nuclei and fibrillary cytoplasmic processes, with immunoreactivity for S-100 protein. The gastrointestinal stromal tumor (GIST) is characterized by a prominent vascular component, cellular clusters with irregular outlines, and spindle cells with delicate cytoplasmic processes (see Fig 7.18). Solitary fibrous tumor and desmoid fibromatosis are distinguished from leiomyosarcoma by their characteristic immunoprofiles (see Table 16.2) and the absence of nuclear atypia.⁷⁹⁻⁸¹

Schwannoma

Schwannomas are benign tumors of nerve sheath origin that primarily affect adults. They have a wide anatomic distribution but usually involve the limbs, head and neck, retroperitoneum, and posterior mediastinum.

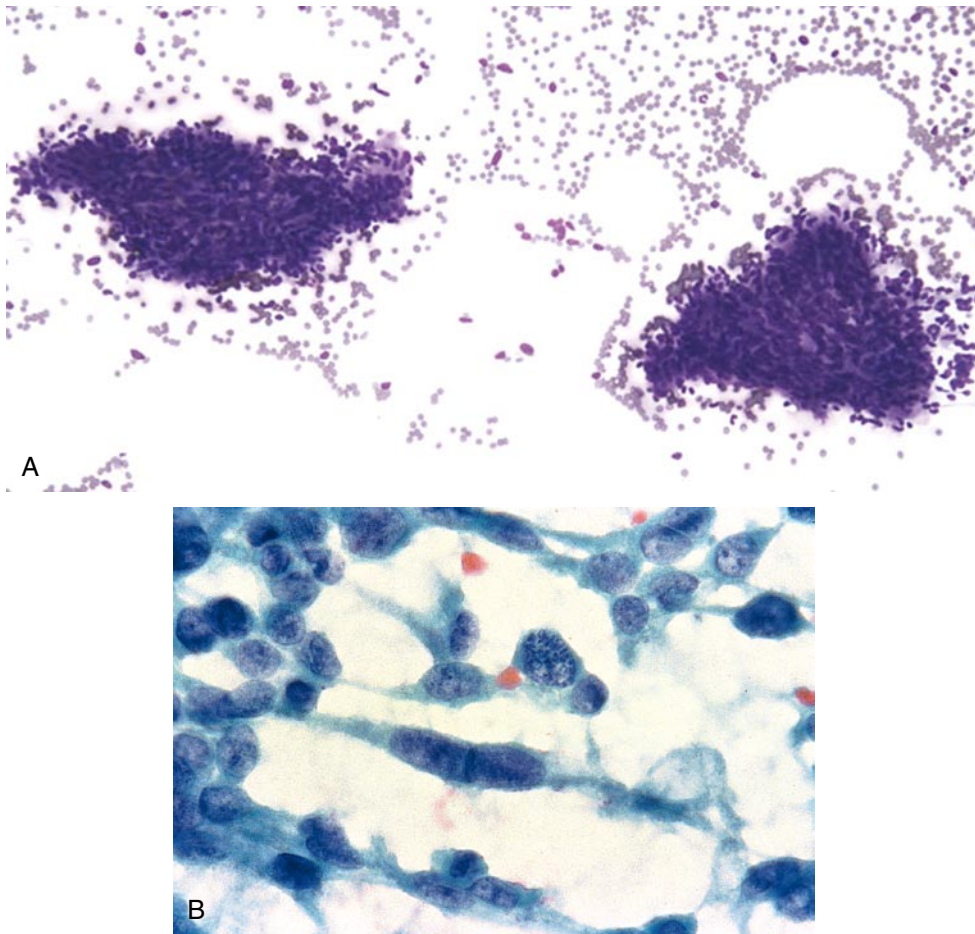


Figure 16.18 Leiomyosarcoma. A, Spindle cells are packed in cohesive tissue fragments with well-circumscribed, rigid edges (Romanowsky stain). B, Nuclei are hyperchromatic, with finely textured chromatin in lower-grade tumors and more coarsely clumped chromatin in the higher-grade neoplasms (Papanicolaou stain).

CYTOMORPHOLOGY OF SCHWANNOMA:

- large, cohesive fragments
- wavy, “fishhook” nuclei
- pointed nuclear ends
- nuclear palisading
- filamentous cytoplasm

Schwannomas often elicit pain during aspiration. Smears contain large tissue fragments that have irregular rather than sharp edges and vary greatly in their cellularity—they can be highly or only sparsely cellular. Antoni A and Antoni B areas can be hard to distinguish with smears. Verocay bodies (palisades of nuclei alternating with anucleate expanses of fibrillar cytoplasm) are present and are especially well-seen on cell block preparations. The elongated, spindle-shaped cells, arranged in syncytium-like fragments, have minimally atypical nuclei that range in shape from oval to wavy to fishhook-like and taper to pointed ends (Fig. 16.19A). In “ancient” schwannomas, nuclear pleomorphism is marked, but focal and scattered. Cytoplasm is abundant

and filamentous (Fig. 16.19B). Mitotic figures are rarely seen.^{1,14,20,42,82} Aspirates from cystic, myxoid, or densely collagenous schwannomas yield few cells and are often non-diagnostic.

DIFFERENTIAL DIAGNOSIS OF SCHWANNOMA:

- spindle cell lipoma
- malignant peripheral nerve sheath tumor (MPNST), low grade
- leiomyosarcoma, low grade
- solitary fibrous tumor
- neurofibroma
- desmoid fibromatosis

A schwannoma often raises the possibility of a malignancy because the rather thick aspirated tissue fragments appear cellular on smears, particularly in the cellular schwannoma variant. This erroneous impression of malignancy is heightened even further with an ancient schwannoma and its large, bizarre nuclei. Diffuse and

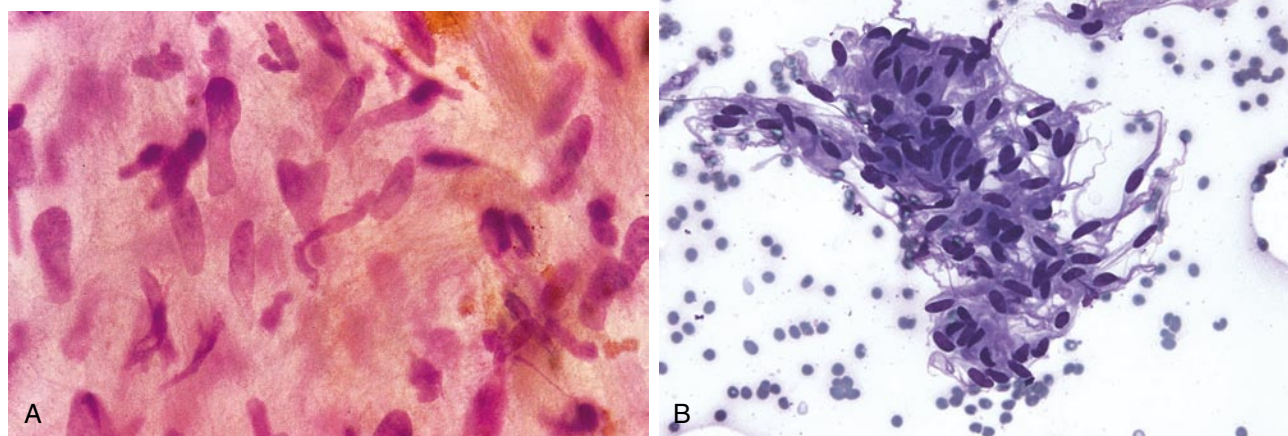


Figure 16.19 Schwannoma. A, The cells of a benign schwannoma have a syncytium-like appearance, with indistinct cell borders (Papanicolaou stain). B, The neoplastic cells have abundant filamentous cytoplasm and a wavy nucleus (Romanowsky stain).

strong immunoreactivity for S-100 protein is characteristic of schwannomas and can be helpful in distinguishing it from malignancies like a low-grade malignant peripheral nerve sheath tumor (MPNST). An MPNST stains only focally or not at all for S-100 protein. A spindle cell lipoma can be distinguished from schwannoma by the presence of mature adipose tissue and its positivity for CD34 in spindle cells. A solitary fibrous tumor has more complex cellular clusters with ropy collagen fibers and bland spindle cells with oval rather than wavy nuclei (see Fig. 16.25). Immunoreactivity for CD34, not S-100 protein, is also helpful. Neurofibroma contains spindle fibroblasts in addition to wavy Schwann cells, and a myxoid background is a common finding. Desmoid fibromatosis yields less cellular smears, with more abundant collagenous stroma.⁸²⁻⁸⁴

Malignant Peripheral Nerve Sheath Tumor

MPNSTs occur sporadically or in patients with neurofibromatosis (NF-1). Often arising from a large peripheral nerve or a preexisting neurofibroma, they also occur in soft tissue and over a wide anatomic distribution. The limbs are most commonly affected, followed by the trunk and the head and neck.

CYTOMORPHOLOGY OF MALIGNANT PERIPHERAL NERVE SHEATH TUMOR:

- hypercellular
- increased number of dispersed cells
- comma-shaped nuclei and nuclear pleomorphism with tapered, pointed or rounded ends
- scant fibrillar cytoplasm
- mitotic figures

Cytomorphology varies depending on the grade and subtype. Aspirates are moderately to highly cellular, with variably sized fascicles, isolated cells, and naked nuclei. Occasional rosette-like or pseudoglandular configurations are seen. Tumor cells are relatively large and predominantly spindled to oval, but can be pleomorphic or rounder in higher-grade lesions. Mild to marked nuclear pleomorphism with occasional bizarre giant nuclei, hyperchromasia, and inconspicuous to prominent nucleoli are seen. The nuclear-to-cytoplasmic ratio is increased, and nuclear palisading is rarely seen. The cells have scant, tapering, and fibrillar cytoplasm, and wavy or comma-shaped nuclei (Fig. 16.20). Mitoses are usually readily identified. Necrosis and apoptosis can be present. MPNSTs show only focal and patchy staining for S-100 protein. Up to 50% of cases are S-100-negative. Occasional cases show perineurial differentiation, recognizable by immunoreactivity for EMA.^{14,44,85-87}

DIFFERENTIAL DIAGNOSIS OF MALIGNANT PERIPHERAL NERVE SHEATH TUMOR:

- schwannoma (especially with “ancient” change)
- synovial sarcoma
- leiomyosarcoma
- spindle cell carcinoma

Densely cellular fascicles alternating with hypocellular myxoid zones, geographic necrosis, and heterologous differentiation favor an MPNST. Low-grade MPNSTs can be difficult to distinguish cytologically from benign peripheral nerve sheath tumors like schwannomas and neurofibromas. A traumatic neuroma following the excision of a sarcoma can be mistaken for recurrent sarcoma. The spindle cell component of synovial sarcoma has less morphologic variability and nuclear pleomorphism, but

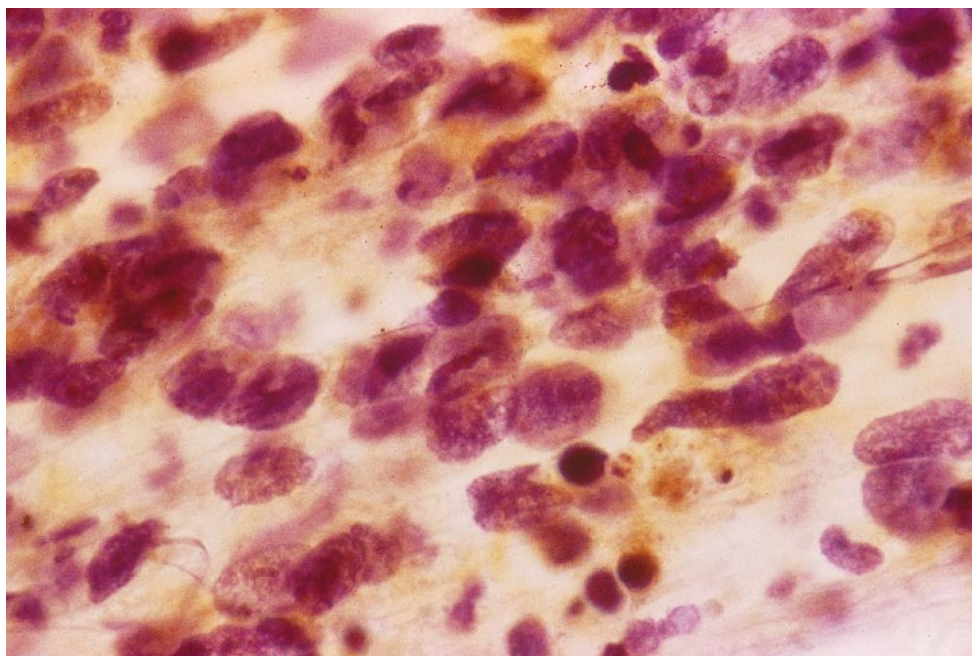


Figure 16.20 Malignant peripheral nerve sheath tumor.

Although some of the elongated, wavy or “fishhook” nuclei of benign nerve sheath tumors are extant, in general, they give way to plumper forms with less pointed ends (Papanicolaou stain).

up to 25% of MPNSTs express cytokeratins. The nuclei of a leiomyosarcoma have blunted or truncated (rather than pointed) ends, and cytoplasm is denser. The cytologic diagnosis of MPNST is difficult in general, even with the help of ancillary studies, because of the lack of reliable specific makers for its line of differentiation and diverse histopathology. Clinical details are often as important (if not more so) for a correct diagnosis of MPNST.^{44,82,87}

Synovial Sarcoma

Synovial sarcoma is a malignant tumor of young adults (aged 10 to 35 years) and accounts for up to 10% of all sarcomas. It has a wide anatomic distribution, and despite its name, it has no particular relationship to synovium. Most are deep-seated and arise in the lower limbs, especially the thigh. Others occur on the trunk and a wide variety of visceral organs. Radiologic evidence of calcification is common.

Histologically, synovial sarcomas can be biphasic (both spindle cell and epithelial components present) or monophasic (either spindle cell or epithelial morphology), and there is a poorly differentiated type that can mimic a small round blue-cell malignancy. Synovial sarcomas are focally immunoreactive for EMA and cytokeratins, especially in areas of epithelial differentiation, but also focally in the spindle cell areas. Up to two thirds of synovial sarcomas express CD99 (O13), and about 30% are positive for S-100 protein. Bcl-2-positivity occurs in most cases. All forms of synovial sarcoma share a reproducible, tumor-specific chromosomal translocation $t(x;18)(p11.2;q11.2)$, which results in two principal fusion genes, *SYT-SSX1* and *SYT-SSX2*. Immunocytochemical studies may not be conclu-

sive in difficult cases. In such cases FISH or RT-PCR are the most effective tools for definitive diagnosis (see [Table 16.1](#) and [Fig 16.23](#)).^{10,13,35,44,88,89}

CYTOMORPHOLOGY OF SYNOVIAL SARCOMA:

- high cellularity
- cell clusters alternating with dispersed cells
- cell clusters with shaggy edges and thin, branching capillaries
- extreme uniformity of cells
- bland, oval nucleus
- scant, delicate, tapering cytoplasm
- occasional epithelial elements
- mitoses
- mast cells

FNAs are moderately to markedly cellular, with occasional fragments of either myxoid or fibrous extracellular matrix. Irrespective of subtype, at low magnification, there is a distinctive pattern of dispersed cells alternating with cohesive cell clusters that contain delicate branching capillaries ([Fig. 16.21](#)). The clusters can be large, with prominent fascicular and whorled growth patterns and shaggy edges. Epithelial structures (glands, acini, tubules, and papillae) can be seen in biphasic tumors, but often the epithelial differentiation is subtle ([Fig. 16.22](#)).^{42,90-93}

Synovial sarcomas are remarkable for the striking uniformity of the cells ([Fig. 16.23A](#)). They are small to medium-sized and round or fusiform. Epithelial differentiation, if present, confers a rounded, polygonal, or even cuboidal to columnar outline, and occasional

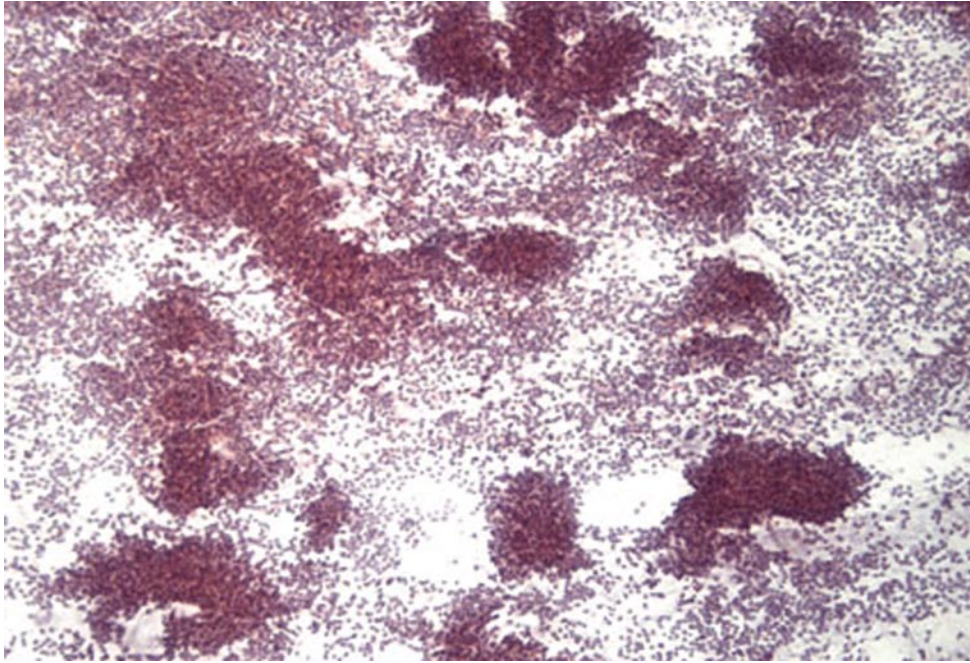


Figure 16.21 Synovial sarcoma. There is a distinctive pattern of dispersed cells alternating with cohesive cell clusters (Papanicolaou stain).

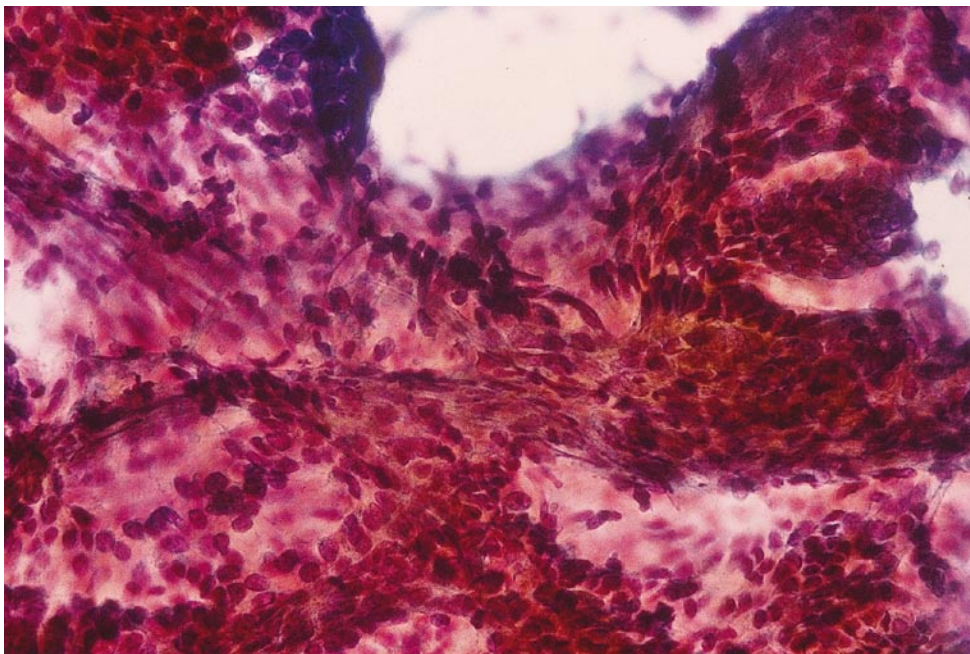


Figure 16.22 Synovial sarcoma, biphasic. Epithelial differentiation is recognized as only a vague palisading of nuclei along the edges of tissue fragments or as clusters of more epithelioid-appearing cells among the spindle cells (Papanicolaou stain).

epithelial cells are flattened as they stretch around three-dimensional cell clusters. The nucleus is bland, round to oval, with finely granular chromatin and a small nucleolus. Nucleoli are occasionally prominent in the epithelial component. In general, the cells have a high nuclear-to-cytoplasmic ratio, with scant, delicate, tapering cytoplasm that is denser and more abundant in the epithelial cells. Mitoses and mast cells are present. Occasionally, necrosis is identified.^{42,90-93}

DIFFERENTIAL DIAGNOSIS OF SYNOVIAL SARCOMA:

- leiomyosarcoma
- MPNST
- solitary fibrous tumor
- Ewing sarcoma/peripheral neuroectodermal tumor (PNET)
- metastatic carcinoma

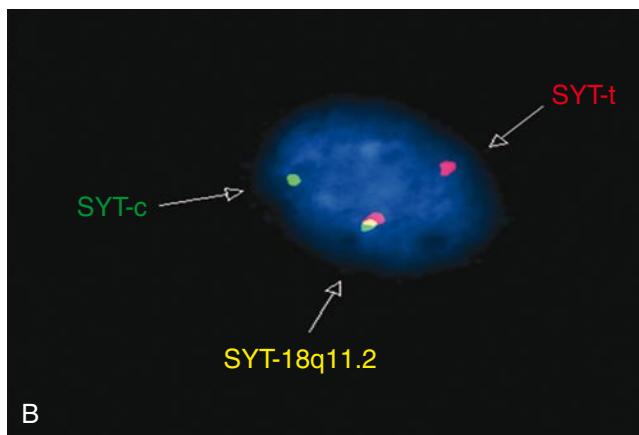
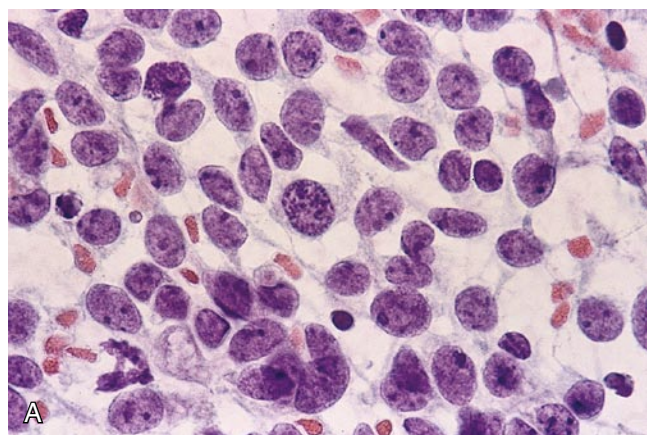


Figure 16.23 Synovial sarcoma, monophasic. A, The oval to round nuclei are remarkably uniform, although rare elongated, blunt-ended, and bent forms are seen (Papanicolaou stain). B, Fluorescence in-situ hybridization (FISH) is often performed with centromeric (SYT-c, green) and telomeric (SYT-t, red) probes that flank the SYT gene breakpoint. There is one normal chromosome (probes adjacent = yellow), but the split-apart green and red signals indicate a translocation on the other chromosome. (Courtesy of Dr. Paola Dal Cin, Brigham and Women's Hospital.)

The nuclei of a leiomyosarcoma have blunted or truncated (rather than rounded) ends, and cytoplasm is denser. Some nuclei of an MPNST are more curved and pointed, and there is greater nuclear pleomorphism. A solitary fibrous tumor has fewer dispersed cells and more complex cellular clusters, with ropy collagen fibers and blood in the background. Immunohistochemistry can be helpful, because a solitary fibrous tumor is usually immunoreactive for CD34 and negative for EMA. At least 20% of synovial sarcomas have a poorly differentiated round cell morphology resembling Ewing sarcoma/peripheral neuroectodermal tumor (PNET; Fig. 16.24). In such cases (indeed, in most cases when a primary

diagnosis is being rendered), immunohistochemistry and genetic analysis are indispensable for confirming the diagnosis of synovial sarcoma (see Fig. 16.23B). Finally, the cells of synovial sarcoma are much more bland and uniform than those of a metastatic sarcomatoid carcinoma.⁹⁰⁻⁹⁴

Solitary Fibrous Tumor

Solitary fibrous tumor (SFT) is a fibroblastic neoplasm that involves the pleura and a wide variety of extrapleural sites, including the peritoneum, mediastinum, retroperitoneum, upper respiratory tract, orbit, deep soft tissues, indeed, almost any human organ. Many SFTs were formerly called hemangiopericytomas. SFT affects adults and presents as a slowly growing mass. The majority of lesions are benign, but about 5% to 10% behave in a malignant fashion.^{44,95} Immunohistochemistry offers valuable support for the diagnosis of an SFT. SFTs are almost invariably positive for CD34 and are only slightly less often immunoreactive for CD99 and Bcl-2. They are negative for S-100 protein, actin, desmin, and the keratins (see Table 16.2).

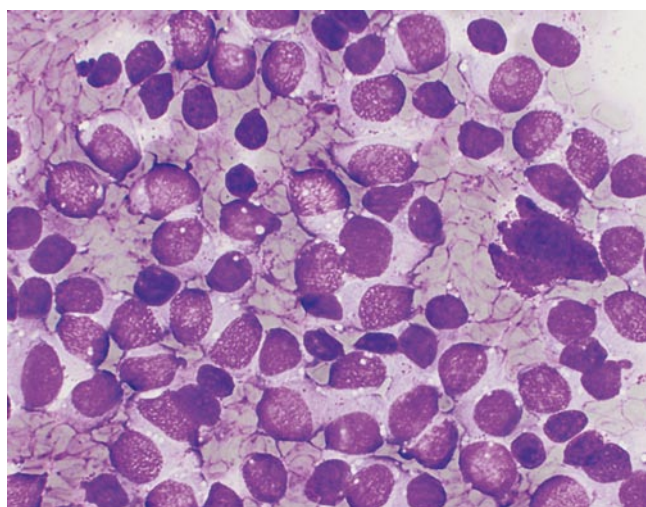


Figure 16.24 Synovial sarcoma, poorly differentiated round-cell type. The tumor cells have a round cell morphology resembling that of a Ewing sarcoma/primitive neuroectodermal tumor (ES/PNET). Mast cells are a characteristic feature (arrow; Romanowsky stain). (Case courtesy of Dr. Bastiaan de Boer, Path West, QEII Medical Center, Nedlands Perth, Australia.)

CYTOMORPHOLOGY OF SOLITARY FIBROUS TUMOR:

- variable yield with bloody background
- both a meshwork of irregular fascicles and individual cells
- bland spindle cells with a fusiform nucleus
- scant elongated cytoplasmic processes
- ropy collagen
- naked nuclei

The aspirate yields scant to moderate cellularity, with a bloody background that contains ropy collagen fibers. Cellular fragments with a meshwork of irregular fascicles alternate with isolated spindle-shaped cells (Fig. 16.25A). The cells are uniformly bland, with a fusiform nucleus, finely dispersed chromatin, an inconspicuous nucleolus, and scant elongated cytoplasm. Naked nuclei are common. Cell block preparations are helpful in capturing the characteristic bland, monomorphic spindle-shaped cells insinuated between collagen bundles (Fig. 16.25B) and hemangiopericytoma-like (i.e., thin, branching, staghorn configuration) vessels.^{1,96-98} Highly cellular clusters with nuclear pleomorphism, prominent nucleoli, necrosis, and noticeable mitotic activity should raise the suspicion

of a malignant SFT.^{97,98} A fat-forming variant of SFT, initially named lipomatous hemangiopericytoma, yields variably prominent adipocytes, even lipoblast-like cells, a diagnostic pitfall that can lead to the misinterpretation of an SFT as any of the benign and malignant lipomatous tumors.^{95,99}

DIFFERENTIAL DIAGNOSIS OF SOLITARY FIBROUS TUMOR:

- MPNST, low-grade
- monophasic synovial sarcoma
- desmoid fibromatosis
- sarcomatoid mesothelioma
- spindle cell thymoma

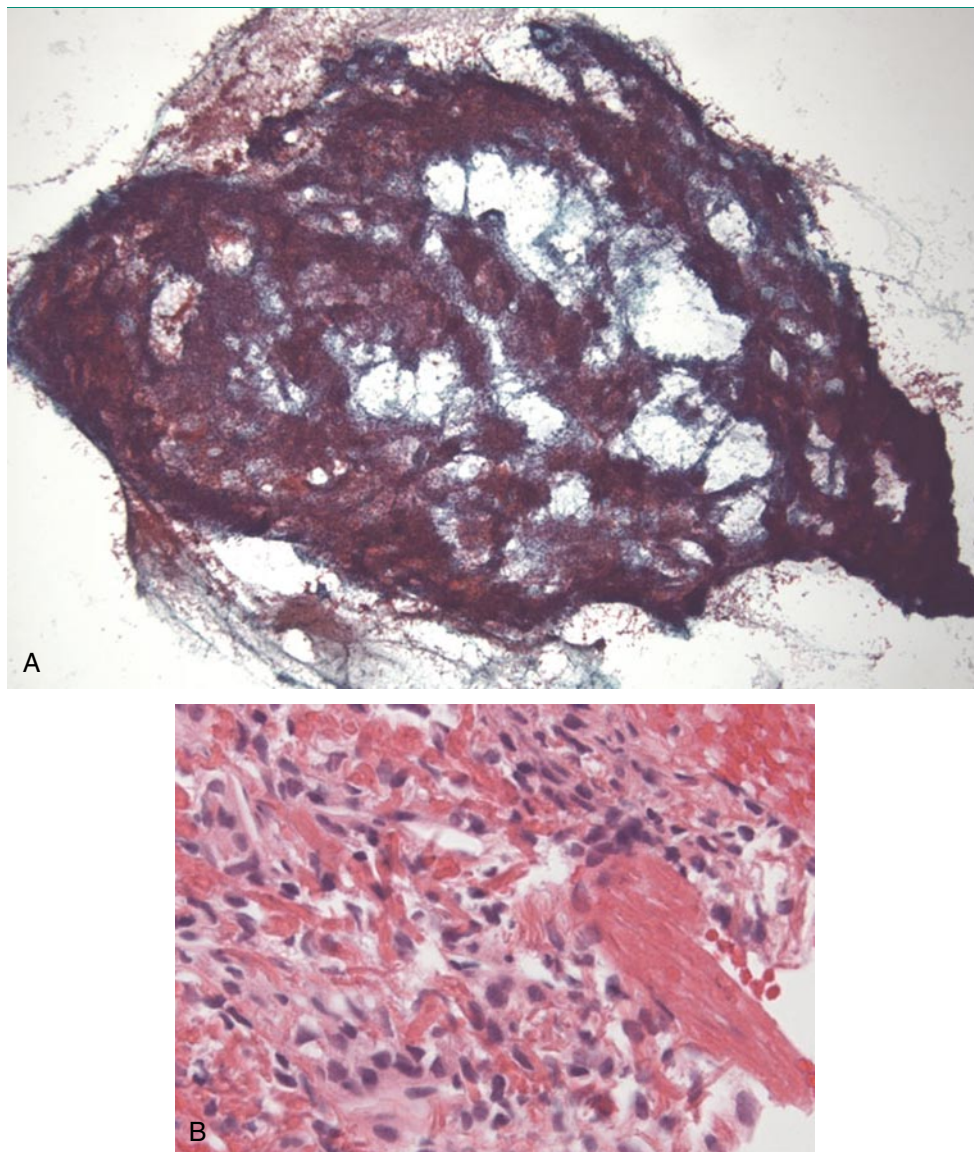


Figure 16.25 Solitary fibrous tumor. A, Cellular fragments with a meshwork of irregular fascicles alternate with isolated spindle-shaped cells (Papanicolaou stain). B, Cell block preparations are helpful for capturing the characteristic bland, monomorphic spindle-shaped cells insinuated between collagen bundles (hematoxylin-eosin [H & E] stain).

MPNST has more nuclear pleomorphism and atypia. Synovial sarcoma exhibits even more striking uniformity in the cell population and cellular arrangements than SFT. Desmoid fibromatosis yields hypocellular smears, with more abundant collagenous stroma. Sarcomatoid mesothelioma and spindle cell thymoma are positive for cytokeratins and negative for CD34.

Fibromatoses

The fibromatoses are a broad spectrum of locally aggressive fibroblastic neoplasms that display an infiltrative growth pattern and can recur but never metastasize. They are divided into two groups based on their location: the superficial or fascial fibromatoses include the palmar, plantar, and penile variants and the deep or desmoid fibromatoses, which include the abdominal, extra-abdominal, and intra-abdominal variants. In childhood and adolescence, desmoid fibromatosis can be the initial manifestation of familial adenomatous polyposis.⁴⁴

CYTOMORPHOLOGY OF FIBROMATOSIS:

- variable cellularity
- bland spindle-shaped and polygonal cells
- fragments of dense collagenous stroma
- oval to elongated nuclei
- rare mitotic activity

FNAs have low but variable cellularity, with loose clusters and isolated bland, spindle-shaped fibroblasts. They have an oval to elongated nucleus with bipolar ends and even chromatin. Some have tapering cytoplasmic processes, whereas others are polygonal or stellate. Rare inflammatory cells are present, but cytologic atypia is absent, and mitotic activity is rare. Fragments of dense collagenous stroma are common. As with the cytomorphology, the immunoprofile is not entirely specific. Positive staining for β -catenin (aberrant nuclear staining) and negative staining for CD34, SMA, and c-Kit support the diagnosis of a fibromatosis in the right clinical setting (see [Table 16.2](#)).^{100,101}

DIFFERENTIAL DIAGNOSIS OF FIBROMATOSIS:

- nodular fasciitis
- low-grade fibromyxoid sarcoma
- scar tissue
- nerve sheath tumor
- smooth muscle tumor
- SFT
- GIST

Nodular fasciitis produces more cellular and pleomorphic smears with a haphazard cellular arrangement and ganglion-like cells. Low-grade fibromyxoid sarcoma has

alternating myxoid and fibrous areas. Scars have less cellularity and greater cohesion. The nuclei in nerve sheath tumors are longer and wavier, those in smooth muscle tumors are blunted, whereas those of fibromatosis have pointed, bipolar ends. SFT and GIST enter the differential diagnosis of intra-abdominal desmoid fibromatosis. These two mimics have more complex, irregular tissue fragments and a more prominent vascular component. Immunohistochemical studies are usually indispensable in classifying these spindle cell neoplasms (see [Table 16.2](#)).

Dermatofibrosarcoma Protuberans

Dermatofibrosarcoma protuberans (DFSP) is a slowly growing fibrohistiocytic tumor of the dermis and subcutis of adults, often preexisting its diagnosis by 5 years or even decades. It occurs mainly on the trunk, proximal extremities, and groin, but a small percentage occur on the scalp or elsewhere in the head and neck region.

CYTOMORPHOLOGY OF DERMATOFIBROSARCOMA PROTUBERANS:

- dense, storiform cell clusters
- entrapped adipose tissue
- minimally pleomorphic spindle cells

Aspirates yield moderately cellular smears composed of dense cellular clusters with a prominent storiform growth pattern. Occasional fragments of metachromatic fibrillary stroma are demonstrable with a Romanowsky stain. Adipose tissue entrapped within fascicles of spindle cells is a useful but inconsistent finding. Isolated fibroblast-like and stellate cells are minimally pleomorphic, with bland, variably sized oval nuclei. The chromatin is fine and homogeneous, and nuclear membranes are smooth and round. Small, distinct nucleoli are usually present. The cytoplasm is moderate in amount, pale, and finely granular. Cell borders are ill-defined within tissue fragments, but bipolar cytoplasmic processes are prominent on dispersed cells.^{102,103} Tumor cells are positive for CD34 and vimentin and are negative for S-100 protein and smooth muscle markers (see also [Table 16.1](#) for cytogenetic alterations).⁴⁴

DIFFERENTIAL DIAGNOSIS OF DERMATOFIBROSARCOMA PROTUBERANS:

- dermatofibroma
- scar
- nodular fasciitis
- smooth muscle tumor
- nerve sheath tumor

Clinical features like the anatomic location and rate of growth distinguish benign fibrohistiocytic lesions

from DFSP, but mitotic rate is variable and unhelpful in these lesions. Scars have less cellularity and greater cohesion with more angulated nuclei. Nodular fasciitis has a more haphazard cellular arrangement, shows more pleomorphic, spindle-shaped and ganglion-like cells, less rounded nuclei, and more prominent nucleoli, often with interspersed neutrophils, eosinophils, and macrophages. The cells of DFSP lack the typical blunt end nuclei of smooth muscle tumors and are negative for smooth muscle markers. Likewise, nerve sheath tumors are excluded by the lack of neural nuclear features and the absence of immunoreactivity for S-100 protein.^{16,102,103}

Inflammatory Myofibroblastic Tumor

Inflammatory myofibroblastic tumor (IMT) is a rare low-grade neoplasm of children and adolescents that was once regarded as a reactive lesion. IMT has a local recurrence rate of 10% to 25%, and metastases occur in just under 5% of cases. Although most common in the lung and liver, it occurs in a variety of other locations (including the soft tissues) and has a predilection for the abdominal cavity. IMTs consist of a moderately cellular, fascicular proliferation of plump, spindle-shaped fibroblastic or myofibroblastic cells with variably atypical nuclei. There is a prominent infiltrate of chronic inflammatory cells, particularly plasma cells. The mitotic rate is variable, and necrosis and calcifications are sometimes present.⁴⁴

CYTOMORPHOLOGY OF INFLAMMATORY MYOFIBROBLASTIC TUMOR:

- inflammatory cells
- mildly pleomorphic spindle cells
- large polygonal cells
- elongated cytoplasmic tails

These tumors yield highly cellular smears with numerous inflammatory cells, including a variable proportion of lymphocytes and plasma cells. Isolated tumor cells are admixed with loose clusters and dense aggregates and are dimorphic: mildly pleomorphic spindle cells and larger, more atypical polygonal cells. The slightly eccentrically placed nuclei are plump and round to oval with finely granular chromatin. The chromatin is coarser and nucleoli more prominent in the larger cells. Some large cells are binucleated or multinucleated. The cells have a low-to-moderate nuclear-to-cytoplasmic ratio. Fine cytoplasmic vacuolization and elongated cytoplasmic tails are typical.^{16,104,105} The tumor cells are positive for actin and occasionally positive for desmin and keratin. Although not entirely specific for IMT, and seen in only 50% of cases, positive immunoreactivity for *anaplastic lymphoma receptor*

tyrosine kinase (ALK), which results from an *ALK* gene rearrangement, can be diagnostically helpful (see [Table 16.1](#)).⁴⁴

Hemangiopericytoma and Fibrosarcoma

Hemangiopericytoma is a controversial entity lacking uniformly accepted diagnostic criteria. There was substantial clinical and pathologic heterogeneity among Stout's original cases. Today, most would be reclassified as other neoplasms, the majority as solitary fibrous tumors. Like hemangiopericytoma, adult fibrosarcoma is best regarded as a diagnosis of exclusion. The majority of the cases diagnosed before the era of immunohistochemistry and electron microscopy (EM) would be reclassified today as either malignant peripheral nerve sheath tumor or monophasic synovial sarcoma. The true fibrosarcomas that remain are the infantile type and the fibrosarcoma arising in DFSP in adults. Neither hemangiopericytoma nor fibrosarcoma is reliably diagnosed by FNA.^{17,44,95,103}

FIBROHISTIOCYTIC NEOPLASMS

The tumors in this group are heterogeneous but bound together morphologically by a mixture of histiocytoid stromal cells, histiocytes, and multinucleated giant cells. They are some of the most commonly targeted neoplasms by FNA. The fibrohistiocytic differentiation of some of these tumors has been challenged over the past 10 years. The so-called pleomorphic and storiform malignant fibrous histiocytoma (MFH), once the most frequently diagnosed sarcoma in adults (40% of adult sarcomas), is now a diagnosis of exclusion and synonymous with *undifferentiated high grade pleomorphic sarcoma* (<5% of adult sarcomas). *Myxofibrosarcoma* (also known as myxoid MFH) and *angiomatoid fibrous histiocytoma* (formerly known as angiomatoid MFH) remain distinct entities. *Myxofibrosarcoma* and *undifferentiated high-grade pleomorphic sarcoma* are discussed in other sections of this chapter. The remaining fibrohistiocytic neoplasms discussed in this section are the localized and diffuse types of giant cell tumor of tendon sheath and the angiomatoid fibrous histiocytoma.^{44,55,69}

Giant Cell Tumor of Tendon Sheath, Localized and Diffuse Types

The localized and diffuse types of giant cell tumor of tendon sheath share similar cellular compositions and cytomorphology but differ in their growth pattern, clinical features, and biological behavior. The *localized type* most commonly presents as a slowly growing, circumscribed, and at least partially encapsulated nodule in close proximity to the synovium of the tendon sheaths of

the hands and feet. It occurs more commonly in females between the ages of 30 and 50 years. The *diffuse type*, also known as *pigmented villonodular synovitis* (PVNS), is a locally destructive lesion with an infiltrative growth pattern, occurring at intra- and extra-articular sites. The diffuse lesion tends to affect younger patients than its localized counterpart. Intra-articular lesions affect predominantly the knee (75% of cases), followed by the hip (15%), ankle, elbow, and shoulder. The extra-articular lesions are usually located in periarticular soft tissue with or without involvement of the adjacent joint.⁴⁴

CYTOMORPHOLOGY OF GIANT CELL TUMOR OF TENDON SHEATH:

- mononuclear histiocyte cells of varying shapes (ovoid, polygonal, and spindle-shaped)
- foamy histiocytes and osteoclast-type giant cells
- extracellular and intracytoplasmic hemosiderin
- moderate anisocytosis and minimal anisokaryosis

Aspirations from localized and diffuse type tumors have moderate to high cellularity. The predominant cell, a mononuclear histiocyte cell, is dispersed as isolated cells and arranged in irregular clusters. These cells have granular to vacuolated cytoplasm and assume a variety of different shapes: ovoid, polygonal, spindle, and stellate. The nuclei, on the other hand, show minimal variation. They are ovoid, with smooth contours, finely granular chromatin, inconspicuous nucleoli, frequent grooves, and occasional intranuclear inclusions

(Fig. 16.26). Binucleation is not uncommon. Although mitotic figures are occasionally present, nuclear atypia and necrosis are quite rare. Variable numbers of foamy histiocytes and osteoclast-type giant cells are admixed within the mononuclear histiocyte cells. The giant cells vary widely in size and in the number of nuclei (3 to 50 or more). The absence of giant cells does not exclude the diagnosis, especially for the diffuse-type tumors. Hemosiderin deposition in mononuclear histiocyte cells and in macrophages is inevitably present.¹⁰⁶⁻¹⁰⁸

DIFFERENTIAL DIAGNOSIS OF GIANT CELL TUMOR OF TENDON SHEATH:

- gouty tophi
- chronic synovitis with synovial hyperplasia
- metastatic melanoma
- giant cell-rich sarcoma

Gouty tophi can have many giant cells but lack mononuclear cells, and the presence of birefringent needle-shaped crystals on polarizing microscopy is diagnostic. Chronic synovitis has more inflammation and sometimes necrosis, but giant cells and hemosiderin deposition are usually not prominent. Metastatic melanoma and giant cell-rich sarcoma have more pleomorphism, nuclear atypia, and necrosis. In difficult cases, immunohistochemistry can be helpful; both the mononuclear cells and the giant cells are positive for the histiocytic marker CD68.

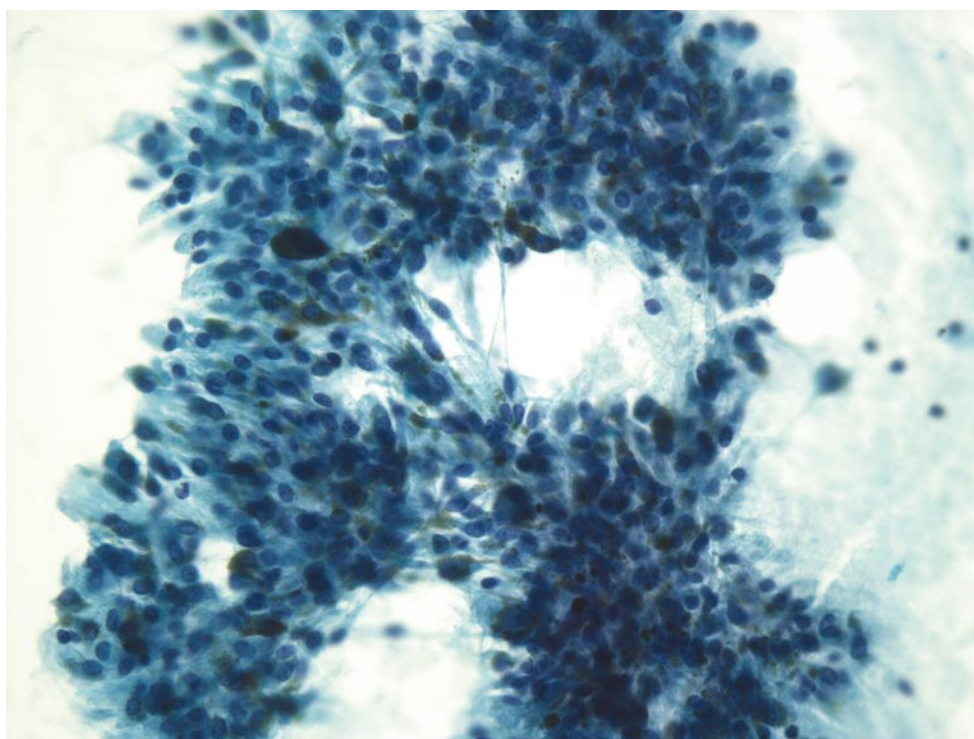


Figure 16.26 Giant cell tumor of tendon sheath. Mononuclear histiocyte cells are arranged in irregular clusters, admixed with foamy histiocytes, giant cells, and prominent hemosiderin deposition (Papanicolaou stain).

Angiomatoid Fibrous Histiocytoma (Angiomatoid Malignant Fibrous Histiocytoma)

Angiomatoid fibrous histiocytoma (AFH), formerly known as angiomatoid MFH, is a reproducibly diagnosable and discrete clinical entity. It is a slowly growing tumor, most often a subcutaneous nodule, of the extremities in children, adolescents, and young adults. It has an indolent behavior; local recurrences occur in 2% to 11%, and metastases in less than 1% of patients.^{44,55}

CYTOMORPHOLOGY OF ANGIOMATOID FIBROUS HISTIOCYTOMA:

- lymphoid cells or bloody background
- clusters of polymorphic cells
- fibroblast-like and histiocyte-like cells
- intracytoplasmic hemosiderin and signs of phagocytosis

Aspirates are moderately to highly cellular, with clusters of mesenchymal cells and inflammatory cells (mainly lymphocytes, few plasma cells, and occasional eosinophils). The two types of mesenchymal cells comprise this tumor: large, histiocyte-like cells with irregular nuclei, prominent nucleoli, and abundant eosinophilic and fragile cytoplasm; and elongated fibroblast-like cells with uniform oval nuclei and vesicular chromatin. Capillary endothelial cells, multinucleated giant cells, hemorrhage, dispersed cells, and rare stromal fragments are also present. The histiocyte-like cells may contain hemosiderin, cellular debris, or pyknotic nuclei.¹⁰⁸⁻¹¹⁰

The histiocyte-like cells are positive for muscle actin (HHF-35), vimentin, EMA, and CD68. Immunoreactivity for desmin is present in 40% to 50% of cases. Tumor cells are negative for smooth muscle actin, cytokeratins, and S-100 protein. Most interesting is the recent recognition that many cases of AFH harbor chromosomal translocations with *EWS-CREB1*, *EWS-ATF1*, and *FUS-ATF1* fusions (see [Table 16.1](#) and [Fig. 16.28](#)).^{44,111}

ROUND CELL NEOPLASMS

The round cell neoplasms are primarily tumors of young patients and account for the majority of solid malignancies in the pediatric age group. Most are high-grade malignancies. Great diagnostic success is attainable in this group of tumors using FNA. The sensitivity and specificity of FNA for the round cell neoplasms exceed 90%, and with an adequate specimen, a diagnosis of a specific histologic subtype is accurately rendered in 93% of cases. Accuracy is even higher with the use of ancillary techniques (see [Table 16.1](#) for cytogenetic alterations).^{10,30,32,42}

Neuroblastoma

Neuroblastoma is the third most common malignant tumor of childhood and the most common malignancy in the neonate. The majority of cases are diagnosed before the age of 5 years. Most cases arise in the adrenal gland or along the intra-abdominal portion of the sympathetic chain, but they can occur anywhere along the sympathetic chain.

CYTOMORPHOLOGY OF NEUROBLASTOMA:

- fibrillary matrix
- mostly dyshesive small undifferentiated cells
- rare neurogenic rosettes
- “salt-and-pepper” chromatin
- rare ganglion-like cells

Smears are cellular, with variable amounts of fibrillary, metachromatic matrix (neuropil) in the background and between the cells. Necrotic debris and dystrophic calcifications are sometimes observed. Tumor cells are predominantly isolated; cohesive aggregates are less common. Homer-Wright rosettes are also present. Most cells are uniformly small and undifferentiated in appearance. Those undergoing differentiation are larger and have a moderate amount of cytoplasm. In even better differentiated lesions, Schwannian-type spindle cells are present. The nuclei are round to oval, with only slight irregularities, finely granular (“salt-and-pepper”) chromatin, and small nucleoli. Occasional nuclear molding is noted. In better differentiated lesions, binucleated and multinucleated ganglion-like cells have a more coarsely granular chromatin, prominent nucleoli, and a moderate amount of cytoplasm. Neuroblastomas are invariably positive for neuron specific enolase (NSE) and show occasional staining for neurofilament protein, synaptophysin, and GFAP. In cases with Schwannian differentiation, immunoreactivity for S-100 is seen. Cytogenetic analysis has become essential for prognosis and clinical management (see [Table 16.1](#)).^{10,30,32,42}

Extrasosseous Ewing Sarcoma/Primitive Neuroectodermal Tumor

Ewing sarcoma/primitive neuroectodermal tumor (ES/PNET) is a primitive neoplasm of neural crest origin. It accounts for 6% of all sarcomas in children and typically affects adolescents and young adults between the ages of 10 and 30 years. The tumor presents as a rapidly enlarging, often painful mass of the deep soft tissues, with a predilection for the paravertebral region of the trunk and less commonly, the intercostal regions of the chest wall, retroperitoneum, pelvis, and lower extremities.

CYTOMORPHOLOGY OF EWING SARCOMA/PRIMITIVE NEUROECTODERMAL TUMOR:

- “tigroid” background
- hypercellular smears with dispersed isolated cells
- “light” cells and “dark” cells
- nuclear molding
- vacuolated cytoplasm

The highly cellular smears often have numerous naked nuclei and a “tigroid” background (a spotted or striped appearance as a result of innumerable small cytoplasmic fragments). The cells are predominantly isolated, with scattered small cohesive clusters (Fig. 16.27). Pseudorosettes are a fairly specific but rare finding. The cells of ES/PNET are approximately two to three times the size of a small lymphocyte and have a high nuclear-to-cytoplasmic ratio. Nuclear molding is prominent.^{30,32,42,112-114}

Traditionally, two distinct cell types have been described, but these just represent a mixture of viable and dying tumor cells. The “light” (viable) cells have paler nuclei with finely granular, evenly dispersed chromatin and distinct nuclear membranes with slight contour irregularities. One or two small nucleoli or chromocenters are readily identifiable, as is an increased amount of pale cytoplasm. The “dark” (dying) cells are smaller, with hyperchromatic, irregularly distributed and smudged chromatin, and unappreciable nucleoli. In both cell types, the nucleus is round to oval. No multinucleated or ganglion-type cells are present. A thin rim

of cytoplasm contains small intracytoplasmic vacuoles that contain glycogen. Perinuclear cytoplasmic clearing is seen in some cases. Cell borders are typically ill-defined, and fine, wispy cytoplasmic tags can sometimes be seen on Papanicolaou-stained smears.

Expression of the Mic-2 glycoprotein product is demonstrable by staining with CD99 (O13) or HBA-71, but the diffuse membranous staining pattern is not entirely specific for ES/PNET. The tumor cells show variable immunoreactivity for NSE, β 2-microglobulin, neurofilament protein, Leu-7 (CD57), and chromogranin. They are non-reactive for muscle markers, cytokeratins, leukocyte common antigen, and κ and λ light chains. The cytogenetic or molecular genetic analysis is the most important ancillary study for confirming the diagnosis. The presence of t(11;22)(q24;q12) or the fusion transcript between *EWS/FLI1* gene is diagnostic for tumors in the ES/PNET family. (see Table 16.1, Fig. 16.28).^{30,44,112,115-119}

DIFFERENTIAL DIAGNOSIS OF EWING SARCOMA/PRIMITIVE NEUROECTODERMAL TUMOR:

- rhabdomyosarcoma
- poorly differentiated synovial sarcoma
- desmoplastic small round cell tumor
- precursor lymphoblastic leukemia or lymphoma
- small cell carcinoma

The differential diagnosis includes all small round cell neoplasms, but most importantly rhabdomyosar-

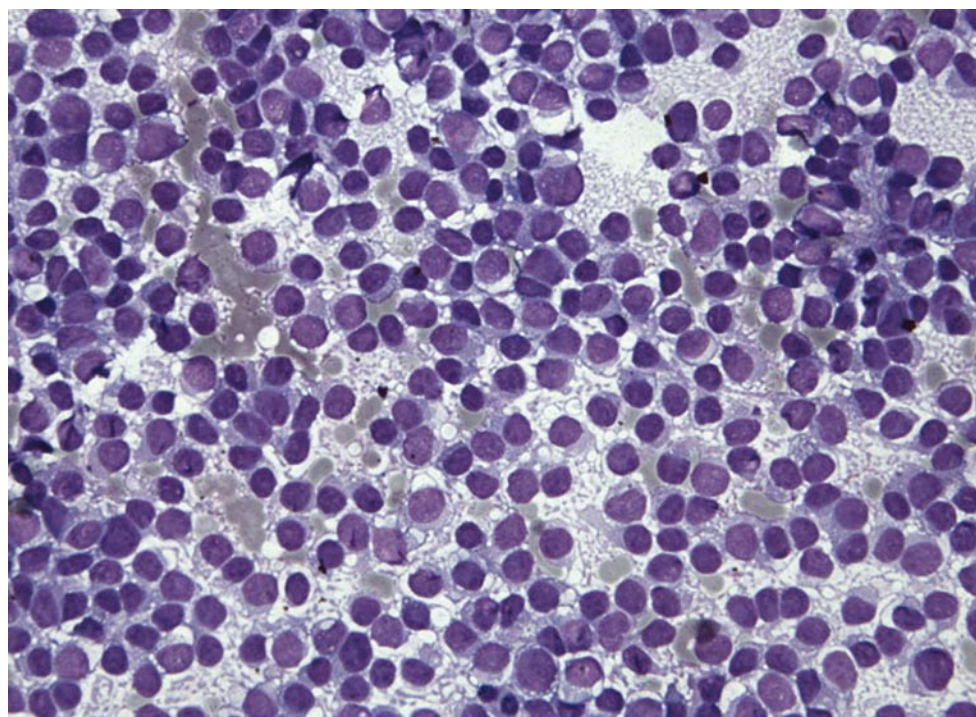


Figure 16.27 Ewing sarcoma/primitive neuroectodermal tumor (ES/PNET). The tigroid background, characteristic light and dark cells, and the occasional cytoplasmic vacuoles are better appreciated in air-dried Romanowsky-stained preparations (Romanowsky stain).

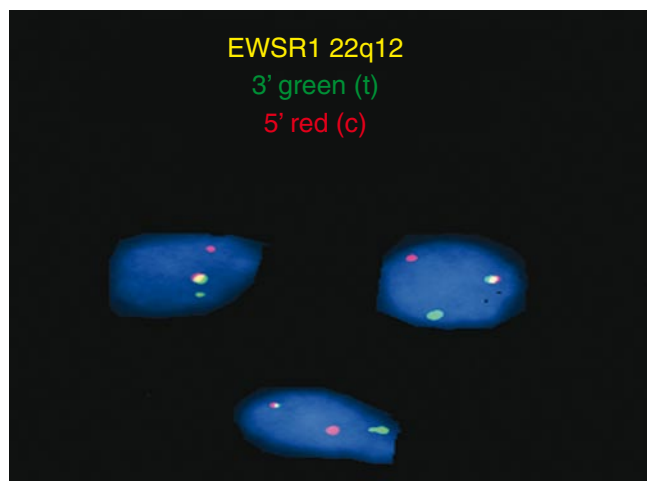


Figure 16.28 Ewing sarcoma/primitive neuroectodermal tumor (ES/PNET). Fluorescence in-situ hybridization (FISH) with centromeric (red) and telomeric (green) probes that flank the *EWS* gene breakpoint show one normal chromosome (probes adjacent = yellow), but the split-apart green and red signals indicate a translocation on the other chromosome in all three cells. FISH cannot distinguish the *EWS* gene rearrangements in ES/PNET, desmoplastic small round cell tumor (DSRCT), clear cell sarcoma, angiomatoid fibrous histiocytoma, and extraskeletal myxoid chondrosarcoma, but these tumors are morphologically and immunophenotypically distinct. (Courtesy of Dr. Paola Dal Cin, Brigham and Women's Hospital, Boston.)

coma (RMS), synovial sarcoma, desmoplastic small round cell tumor (DSRCT), small cell carcinoma, and precursor lymphoblastic leukemia or lymphoma. RMS usually has larger rhabdomyoblasts with more abundant, denser cytoplasm, and multinucleated and spindle-shaped cells in addition to small rounded cells. Positive staining for EMA or cytokeratins along with cytogenetic analysis aids in the correct recognition of the poorly differentiated synovial sarcoma. DSRCT has smaller and more cohesive cells and exhibits a polyphenotypic immunoprofile. Small cell carcinomas have more prominent nuclear molding, mitoses, and necrosis. The cells of precursor lymphoblastic leukemia or lymphoma are accompanied by lymphoglandular bodies and are immunoreactive for lymphoid markers.^{112,114,120,121}

Desmoplastic Small Round Cell Tumor

Desmoplastic Small Round Cell Tumor (DSRCT) is a neoplasm of uncertain histogenesis with a striking predilection for forming large and small tumor masses on the serosal surfaces. Most cases are intra-abdominal, but the tumor can involve the pelvis, retroperitoneum, scrotum, and pleura. DSRCT affects mainly male adolescents and young adults between the ages of 15 and 35 years.

CYTOMORPHOLOGY OF DESMOPLASTIC SMALL ROUND CELL TUMOR:

- sheets and clusters of small to intermediate-sized cells
- fragments of desmoplastic stroma
- uniformly round to oval cells
- nuclear molding

Smears are variably cellular, with sheets and clusters of loosely cohesive, small to intermediate-sized cells. The groups often recapitulate the irregular shapes of the nests and islands seen in histologic sections. Occasional isolated cells are present among fragments of variably cellular and collagenous (“desmoplastic”) stroma. The tumor cells are uniformly undifferentiated and predominantly round to oval, with rare polygonal or spindled forms (Fig. 16.29). Nuclei are hyperchromatic, with finely granular chromatin and inconspicuous nucleoli. Nuclear molding can be striking, particularly at the edges of groups. The scanty cytoplasm stains palely amphophilic to eosinophilic, and occasional “rhabdoid” cells are present. Tumor cells in postchemotherapy cases can have a different cytomorphology, with isolated larger cells with conspicuous nucleoli.¹²²

The differential diagnosis is similar to that for ES/PNET. DSRCT cells demonstrate a peculiar but characteristic polyphenotypic differentiation, with positive immunoreactivity for low-molecular-weight cytokeratins, EMA, neuroendocrine markers, and desmin, the latter in a characteristic dotlike pattern. They are also positive for Wilms tumor (WT1) and variably positive for CD99. DSRCT demonstrates a specific cytogenetic abnormality with t(11;22)(p13;q12) involving the *EWS* gene on 22q12 and the *WT1* gene on 11p13 (Fig. 16.28).^{32,122-124}

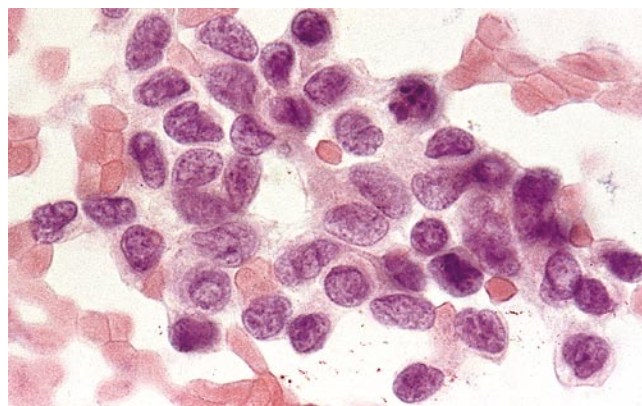


Figure 16.29 Desmoplastic small round cell tumor (DSRCT). This tumor differs morphologically from the other round cell neoplasms because its cells retain some cohesiveness and are rarely dispersed as isolated cells (Papanicolaou stain).

Embryonal Rhabdomyosarcoma

RMS has three main subtypes: *embryonal* (including the botryoid and spindle cell variants), *alveolar*, and *pleomorphic* (discussed in Chapter 4). The embryonal and alveolar forms occur mainly in children, whereas pleomorphic RMSs occur almost exclusively in adults. RMS is the most common sarcoma of childhood, and embryonal RMS accounts for the majority in children (60%). The most common sites are the head and neck region (including the orbit and meninges), the genitourinary tract, and the trunk. Because the distinction between its two principal forms, embryonal and alveolar, has significant prognostic implication, and cytomorphology is usually insufficient for this distinction, ancillary diagnostic methods, especially cytogenetic or molecular studies, have become the standard of care.^{1,44,125}

CYTOMORPHOLOGY OF EMBRYONAL RHABDOMYOSARCOMA:

- predominantly isolated round or spindle cells
- cellular pleomorphism
- variable number of rhabdomyoblasts
- occasional inclusion-like cytoplasmic condensation (myogenic differentiation)

Aspirates are moderately to highly cellular, composed predominantly of isolated cells, with occasional loose clusters, and uncommonly, a frothy “tigroid” or myxoid background. The tumor cells vary from small-sized to intermediate-sized and are round, polygonal, or spindle-shaped. Larger cells show rhabdomyoblastic differentiation, with elongated, strap- or tadpole-shaped cytoplasm and eccentric nuclei. Anisonucleosis and cellular pleomorphism are especially pronounced among the larger cells. Nuclei have dense hyperchromatic chromatin and irregular membranes. Occasional cells have inclusion-like cytoplasmic condensations indicating myogenic differentiation. Cytoplasmic cross-striations are rarely seen.^{32,125-129}

Embryonal RMS cells show myogenic differentiation manifested by positive immunoreactivity for desmin, muscle-specific actin, and for protein products of specific myogenic nuclear transcription factors myo-D1 and myogenin (myf-4).

Alveolar Rhabdomyosarcoma

Alveolar RMS, a subtype with unfavorable prognosis, is a tumor of older children that occurs most frequently in adolescents. The limbs, head and neck region, and trunk are the most common sites.

CYTOMORPHOLOGY OF ALVEOLAR RHABDOMYOSARCOMA:

- larger, uniformly round to polygonal cells
- variable number of rhabdomyoblasts
- multinucleated tumor giant cells with wreath-like nuclei
- mitoses

Aspirates are highly cellular and infrequently have a “tigroid” background. Most cells are undifferentiated, with uniformly round to polygonal outlines (Fig. 16.30). Compared to the tumor cells of the embryonal variant, alveolar RMS cells are rounder, with larger and more irregular nuclei. Variable number of rhabdomyoblasts and multinucleated giant tumor cells, with or without “wreath-like” nuclei, are helpful diagnostic features when present. Mitoses are common.^{1,125,127,129}

DIFFERENTIAL DIAGNOSIS OF ALVEOLAR RMS:

- ES/PNET
- neuroblastoma
- poorly differentiated synovial sarcoma
- precursor lymphoblastic lymphoma or leukemia
- rhabdoid tumor

Like its embryonal cousin, alveolar RMS is immunoreactive for desmin, muscle-specific actin, myo-D1, and myogenin. In recent years, cytogenetic or molecular genetic analysis have become essential for confirming and refining the diagnosis of RMS (see also Table 16.1 for cytogenetic alterations).^{44,125}

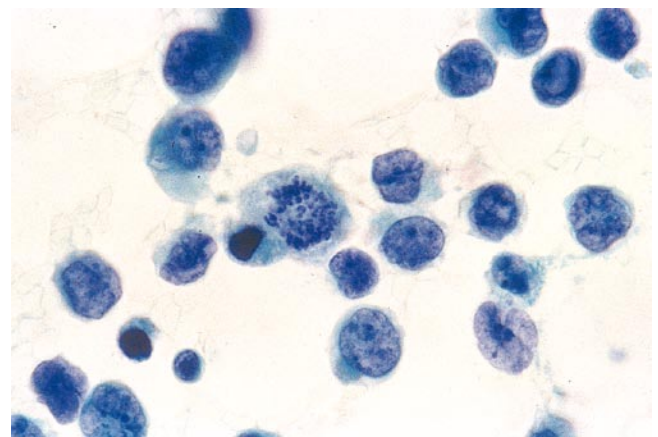


Figure 16.30 Alveolar rhabdomyosarcoma (RMS). The cells disperse individually and are generally larger and more uniformly round to polygonal than those seen in embryonal rhabdomyosarcoma (Papanicolaou stain).

Extraskelatal Mesenchymal Chondrosarcoma

Mesenchymal chondrosarcoma (MC) is an uncommon cartilaginous tumor that primarily occurs in adolescents and young adults, accounting for about 1% to 2% of all chondrosarcomas. Approximately one third are extraskelatal and affect the soft tissues of the orbit, cranial and spinal meninges, and lower limbs. Rare cases occur in the mediastinum, hand musculature, retroperitoneum, kidney, and lung.^{44,108}

CYTOMORPHOLOGY OF EXTRASKELETAL MESENCHYMAL CHONDROSARCOMA:

- cartilaginous matrix
- two components: small round primitive cells and well-differentiated cartilaginous tissue
- cellular uniformity in primitive component
- frequent mitoses

Smears are hypercellular and remarkable for two distinct components. One is well-differentiated, hyaline cartilage, with polygonal cells that have vacuolated cytoplasm, a round nucleus, granular chromatin, and a prominent nucleolus. This component has features of a well-differentiated chondrosarcoma. The second is made up of primitive small round cells that aggregate into compact groups, with some isolated cells. The primitive cells are uniform in appearance, each with a round to oval nucleus, granular chromatin, a thick nuclear membrane, an inconspicuous nucleolus, and scant cytoplasm. Mitoses are frequent and often atypical. There can be necrosis and inflammation. A concurrent core biopsy can provide valuable information by preserving the distinctive architectural relationship between the two components. The tumor is S-100-positive in the chondroblastic component. The small round cells can be positive for CD99.^{108,130-132}

EPITHELIOID NEOPLASMS

Epithelioid soft tissue neoplasms are tumors composed of medium-sized or large, round or polygonal cells with ample cytoplasm. Metastatic carcinoma and melanoma are always included in the differential diagnosis. Epithelioid neoplasms yield cellular smears with cohesive tumor cell aggregates and numerous individually dispersed neoplastic cells.⁹

Epithelioid Sarcoma

Epithelioid sarcoma is an extraordinarily rare malignancy of adolescents and young adults with a striking predilection for males. It affects the distal extremities, especially the hands and wrists, and less commonly

involves the lower legs, buttocks, and thighs. The tumors are generally superficial, involving the dermis and subcutis, or tendo-aponeurotic. They present as slowly growing masses often of long duration. Pain and ulceration are frequent.⁴⁴ A minority of epithelioid sarcomas are termed *proximal type* and may represent a unique subtype of epithelioid sarcoma. These tumors have aberrations of the *INI1* gene on 22q, which results in the loss of INI1 protein expression in some cases.

CYTOMORPHOLOGY OF EPITHELIOID SARCOMA:

- granuloma-like structures
- necroinflammatory debris
- numerous dispersed medium-sized or large, round to polygonal cells
- eccentrically placed nucleus
- dense cytoplasm

Smears are moderately cellular, with a background of blood, necroinflammatory debris, and occasional granuloma-like structures. A dominant pattern of isolated cells alternates with small, loosely cohesive clusters of cells. The malignant cells are round or polygonal, occasionally spindle-shaped, and they vary only slightly in size. Mild to moderate nuclear pleomorphism is appreciated in some cases. The nuclei are large, round, eccentrically placed, and hyperchromatic, with vesicular, occasionally clumped chromatin and one or more prominent nucleoli. Binucleation and multinucleation are common. The moderate to abundant cytoplasm is dense and can contain vacuoles (Fig. 16.31A-D). Pale perinuclear zones in some cells correspond to accumulations of intermediate filaments. Interstitial spaces define cytoplasmic borders. Normal and atypical mitoses are common. Necrosis is present either as a thin background film or as dense blocks with peripherally palisaded tumor cells (the aforementioned granuloma-like structures).^{133,134}

DIFFERENTIAL DIAGNOSIS OF EPITHELIOID SARCOMA:

- metastatic carcinoma
- synovial sarcoma
- epithelioid hemangioendothelioma
- epithelioid angiosarcoma
- metastatic melanoma
- granulomatous inflammation

Even with the help of immunohistochemistry, epithelioid sarcoma is difficult to distinguish from a metastatic carcinoma. It is diffusely and strongly positive for cytokeratins and EMA. The clinical presentation of an extremity mass in a young patient is an important clue to the correct diagnosis. Synovial sarcoma, which also stains for

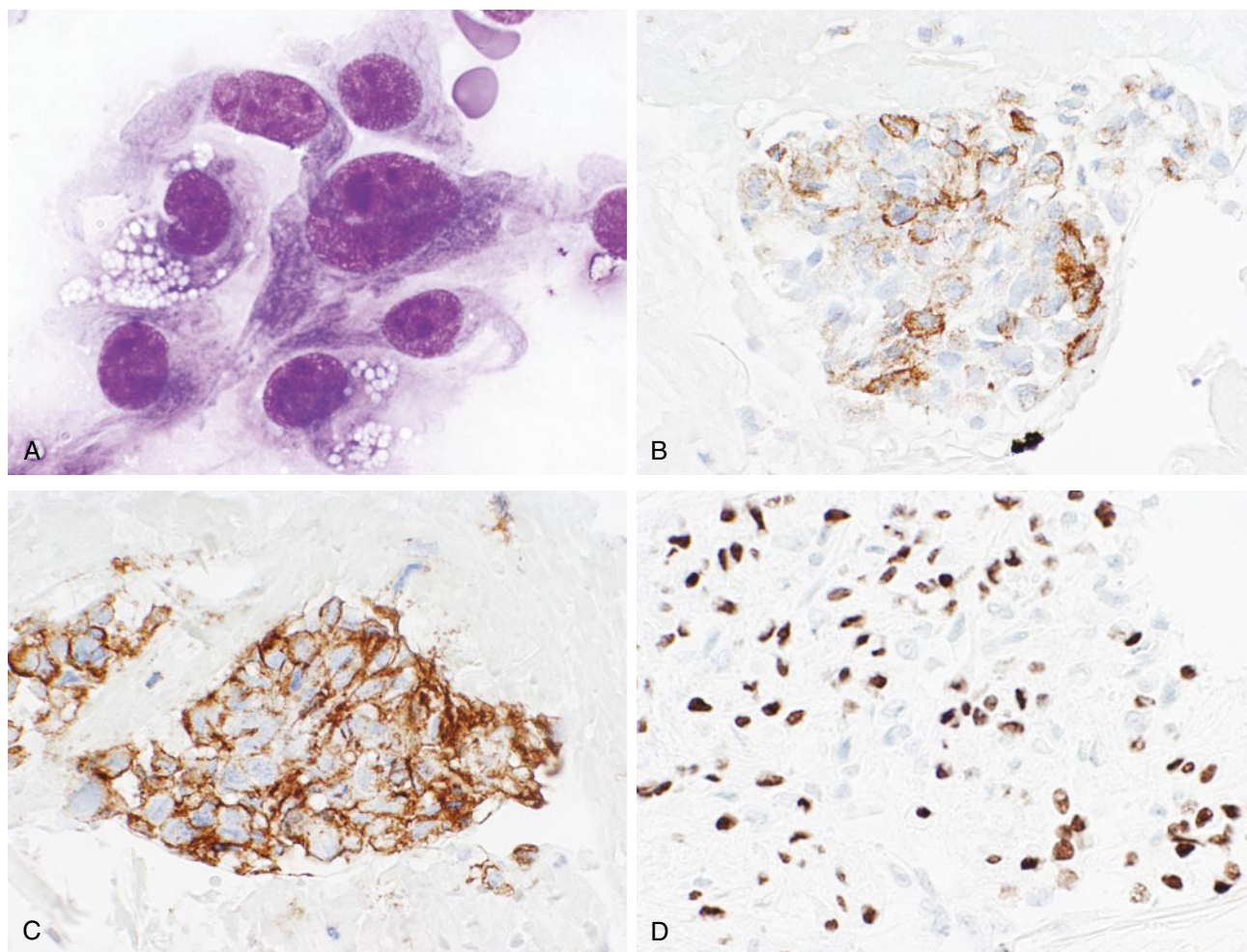


Figure 16.31 Epithelioid sarcoma. A, The smear shows polygonal, rounded, and spindle-shaped cells, some with cytoplasmic vacuoles. (Romanowsky stain). B, Tumor cells are epithelial membrane antigen (EMA)-positive. C, Tumor cells are CD34-positive. D, There is loss of INI1 in a subset of tumor cells, which is characteristic of the proximal type of epithelioid sarcoma.

cytokeratins and EMA has smaller, oval, and less pleomorphic cells. Although epithelioid sarcoma is negative for most vascular markers like CD31 and factor VIII, it is often positive for CD34, a potential problem in distinguishing it from an epithelioid hemangioendothelioma (see Fig. 4.15) and epithelioid angiosarcoma (see Fig. 2.32). The lack of staining for HMB-45 and S-100 protein excludes metastatic malignant melanoma. True granulomas lack cytologic atypia and are negative for cytokeratin.^{44,133-135}

Clear Cell Sarcoma (Malignant Melanoma of Soft Tissues)

Clear cell sarcoma (also known as malignant melanoma of soft tissues) is a sarcoma of neural crest origin with melanocytic differentiation. It accounts for less than 1% of all soft tissue neoplasms. The tumor affects primarily adolescents and young adults between the ages of 20 and 40 years. Presenting as a slowly growing, often painful mass of the deep tissues of the extremities, it is usually closely associated with fascia, tendons, or aponeuroses. Most

lesions measure less than 5 cm. Unless an amputation is performed, repeated local recurrence is common.⁴⁴

CYTOMORPHOLOGY OF CLEAR CELL SARCOMA:

- dispersed round, polygonal, or spindle-shaped cells
- prominent nucleolus
- “wreath-like” multinucleated giant cells
- intranuclear cytoplasmic pseudoinclusions
- clear to pale staining cytoplasm

Cytologic preparations are moderately to highly cellular, with a remarkably clean background. The cells are mostly isolated, with occasional small clusters. They vary in size and shape and can be round, polygonal, and fusiform. Most nuclei are uniform in size, large, round to oval, and eccentrically placed (Fig. 16.32). They are slightly hyperchromatic with finely granular, irregularly distributed, vesicular chromatin. The nucleolus is usually

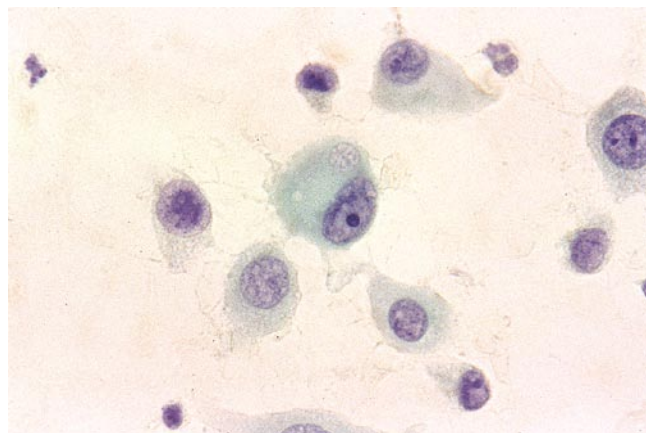


Figure 16.32 Malignant melanoma of soft tissues. Cell borders are clearly defined, and delicate cytoplasmic processes extend from a number of the cells (Papanicolaou stain).

central, prominent, and solitary, but two or three smaller nucleoli can be seen. Intranuclear cytoplasmic pseudoinclusions are almost invariably present. Binucleated cells are ubiquitous, and wreath-like multinucleated giant cells are rare but diagnostically important. The moderately abundant cytoplasm is clear and pale, even imperceptible in some cells, and rarely finely vacuolated. Most of the scant intracytoplasmic melanin is found in macrophages. Occasional mitoses are seen, but necrosis is not a typical feature. Most cases are strongly positive for S-100 protein and HMB-45. Clinical and cytogenetic findings (see Table 16.1, Fig. 16.28) help distinguish it from metastatic cutaneous melanoma.^{27,136-138}

Alveolar Soft Part Sarcoma

Accounting for less than 1% of all sarcomas, alveolar soft part sarcoma (ASPS) is a tumor of older adolescents and young adults, with a slight female predominance. It is a slowly growing, deep-seated, painless mass most common in the lower extremities or limb girdle, especially the anterior thigh. In children, the head and neck region is mainly affected.⁴⁴ These tumors are negative for cytokeratins, EMA, chromogranin, and synaptophysin. ASPS has a characteristic genetic anomaly, an unbalanced (X;17) translocation (see Table 16.1) that results in a *transcription factor E3(TFE3)*-*ASPL* fusion gene and nuclear overexpression of TFE3 protein.

CYTOMORPHOLOGY OF ALVEOLAR SOFT PART SARCOMA:

- naked nuclei
- pseudoacinar or pseudoalveolar arrangements
- large, round to polygonal cells
- prominent nucleolus
- abundant granular and fragile cytoplasm
- intracytoplasmic and extracellular crystals

Aspirates show slight to moderate cellularity, with numerous naked nuclei. Often, a background of disrupted cytoplasmic contents and capillary networks devoid of tumor cells is seen. The cells are largely dyshesive but sometimes aggregate in large groups. Pseudoacinar or pseudoalveolar arrangements are surrounded by a thin vascular structure reminiscent of the endothelial capping seen with some hepatocellular carcinomas (HCCs). The cells are uniformly round to polygonal with some variation in size. The mildly pleomorphic, hyperchromatic, large nuclei are round to oval, with vesicular, finely stippled chromatin, smooth nuclear contours, and a central, prominent, round nucleolus. The fragile cytoplasm is abundant and finely granular. Intracytoplasmic and extracellular periodic acid-Schiff (PAS)-positive rhomboid crystals are a helpful feature but are rarely identified. Mitotic activity is low.

DIFFERENTIAL DIAGNOSIS OF ALVEOLAR SOFT PART SARCOMA:

- granular cell tumor
- rhabdomyoma
- paraganglioma
- renal cell carcinoma
- endocrine neoplasm

Renal cell carcinoma, paraganglioma, rhabdomyoma, endocrine neoplasms, and granular cell tumor resemble ASPS, but all lack rhomboid crystals and have different immunoprofiles.¹³⁹⁻¹⁴² A specific diagnosis can usually be made based on its distinctive cytomorphology, immunoprofile, and characteristic ultrastructural features.^{139,143}

Epithelioid Hemangioendothelioma

Epithelioid hemangioendothelioma (EHE) is a rare vascular tumor of intermediate malignancy, seen almost exclusively in adults over the age of 30 years. It occurs in the lung and liver, but it can arise in other sites like bone and soft tissues, skin, peritoneum, lymph nodes, and (rarely) the brain and meninges. About half arise from a large-sized or medium-sized vessel. Multicentricity is frequent. Lung and liver primaries occur more commonly in females. Local recurrence and rare distant metastasis are sequelae of aggressive forms.⁴⁴

CYTOMORPHOLOGY OF EPITHELIOID HEMANGIOENDOTHELIOMA:

- bloody background
- round to polygonal plasmacytoid cells
- only slight nuclear pleomorphism and frequent binucleation
- abundant cytoplasm with occasional intracytoplasmic lumina
- few mitoses

Smears are highly cellular, with a substantially bloody background and fragments of metachromatic, hyaline or chondroid stroma. Although predominantly isolated, the cells can form small, loose aggregates and rosette-like formations. The cells are monomorphic, with round, oval, or polygonal contours, a plasmacytoid appearance, and abundant, dense eosinophilic cytoplasm (see Fig. 4.14). Rare, sharply demarcated intracytoplasmic lumina (vacuoles) distort the nucleus. Nuclei are round to oval or occasionally reniform or polylobated, with frequent binucleation and no significant nuclear pleomorphism. The chromatin is unevenly distributed, and one or two small nucleoli are seen. Occasional larger cells have hyperchromatic, irregular nuclei with nuclear membrane clefts and protrusions. Mitoses are seldom seen. Tumor cells are strongly and diffusely positive for vascular markers CD31, CD34 and factor VIII-related antigen. Up to 26% are immunoreactive for cytokeratin, but they are negative for EMA. The demonstration of Weibel-Palade bodies by EM in tumor cells is another way to confirm their endothelial origin. They differ from the normal endothelial cells by the presence of abundant intermediate filaments.¹⁴⁴⁻¹⁴⁸

DIFFERENTIAL DIAGNOSIS OF EPITHELIOID HEMANGIOENDOTHELIOMA:

- metastatic carcinoma
- melanoma
- epithelioid sarcoma
- angiosarcoma
- mesothelioma
- epithelioid hemangioma

The tumor is distinguished cytologically from metastatic carcinoma, melanoma, epithelioid sarcoma, and angiosarcoma by its lower degree of nuclear atypia and mitotic activity. EHEs of the lung have a predilection for spreading to the pleural surfaces and clinically mimicking a mesothelioma, not infrequently presenting with a pleural effusion. The similarity extends to morphologic evaluation: EHEs strongly resemble an epithelioid mesothelioma. The distinction rests with immunohistochemistry; tumor cells of epithelioid mesothelioma are positive for mesothelial cell markers (calretinin, WT1) whereas those of EHE are reactive for endothelial cell markers (CD31, CD34, etc.). Epithelioid hemangioma is less commonly multicentric and more obviously vasoformative. It lacks hyaline or chondroid stroma and is often associated with an inflammatory infiltrate.^{44,146,147}

Epithelioid Angiosarcoma

Epithelioid angiosarcoma, another rare but distinctive vascular tumor, has a marked predilection for males, usually adults in mid to late life. It is a rapidly growing

tumor of deep soft tissues, but it can be encountered in a variety of other sites, including the adrenal gland, pleura, pulmonary artery, breast, bone, and vagina.⁴⁴

CYTOMORPHOLOGY OF EPITHELIOID ANGIOSARCOMA:

- extensively bloody background
- large, dyshesive epithelioid cells
- moderate to marked nuclear pleomorphism
- prominent nucleolus
- occasional angioformative structures: pseudo-acini with central erythrocytes or intracytoplasmic lumina containing erythrocytes
- numerous mitoses

Smears are variably cellular depending on the degree of dilution by blood. The mostly dyshesive cells infrequently aggregate into small clusters and sometimes even form pseudoacini. They are large, rounded epithelioid cells with moderately to markedly pleomorphic nuclei and smooth nuclear contours (see Fig. 2.32). Binucleation and especially multinucleation are rare. Mitoses are frequent. The nuclei are eccentrically placed within an abundant, finely granular cytoplasm. Intracytoplasmic hemosiderin is detected in many cases. Angioformative structures (e.g., pseudoacini with central erythrocytes; intracytoplasmic lumina (vacuoles) containing intact or fragmented erythrocytes) are rare but useful findings. Immunocytochemistry reveals immunoreactivity for the vascular markers von Willebrand factor, factor VIII-related antigen, CD31, and CD34 and for cytokeratins in up to 50% of cases. EM is occasionally helpful in demonstrating endothelial differentiation when immunohistochemistry fails, but Weibel-Palade bodies (a specific tubular organelle of normal endothelium) are only rarely identified.^{146,148-152}

Granular Cell Tumor

Granular cell tumors are relatively common, slowly growing neoplasms derived from Schwann cells. With rare exceptions, they are benign tumors. They occur at any site but are most common in the trunk and tongue of middle-aged adults.⁴⁴ Many granular cell tumors are sampled by FNA during the work-up of breast lesions.^{153,154}

CYTOMORPHOLOGY OF GRANULAR CELL TUMOR:

- bare nuclei in a granular background
- uniform cellular appearance
- small nuclei
- abundant granular cytoplasm

Smears show numerous naked nuclei and a finely granular background derived from the abundant, fragile cytoplasm of the tumor cells. Intact cells are uniform and occur as syncytial groups and isolated cells (Fig. 16.33). The small, round to oval nuclei are minimally pleomorphic, with finely granular chromatin and a small but conspicuous nucleolus. Rare intranuclear cytoplasmic invaginations can be seen, and cell borders are typically ill defined. Mitoses and necrosis are not seen in aspirates of benign lesions. Malignant granular cell tumors are exceedingly rare, and it is difficult to definitively separate them from their benign counterparts. The cytologic features that are suggestive of malignancy include spindle cell morphology, cellular pleomorphism, enlarged nucleoli, and mitoses.¹⁵⁵ Tumor cells in both benign and malignant forms show strong cytoplasmic staining for S-100 protein. They are also immunoreactive for other melanocytic markers, such as microphthalmia transcription factor (MITF) and NKI/C3, a melanoma-associated antigen. Interestingly, they are also positive for CD68, inhibin, and calretinin. They are negative for muscle markers, GFAP, HMB-45, and cytokeratins.^{44,153,156}

DIFFERENTIAL DIAGNOSIS OF GRANULAR CELL TUMOR:

- fat necrosis
- Whipple disease
- rhabdomyoma
- ASPS
- granular renal cell carcinoma

Fat necrosis has bean-shaped or oval histiocytes with foamy cytoplasm, multinucleated giant cells, and

other inflammatory cells. A similarly mixed cell population is typical of Whipple disease. Rhabdomyoma may contain multinucleated cells and is negative for S-100 protein. ASPS has prominent nucleoli, frequent binucleation and multinucleation, and pseudoacinar or pseudoalveolar arrangements. Granular renal cell carcinomas usually have some cells with clear cytoplasm.^{153,156}

PLEOMORPHIC NEOPLASMS

Many pleomorphic soft tissue tumors are poorly differentiated or dedifferentiated forms of soft tissue tumors that have a clear line of differentiation (e.g., forming adipose tissue, skeletal muscle, etc.). Some have been discussed previously (e.g., pleomorphic and dedifferentiated liposarcomas; pleomorphic RMS). With the exceptions of atypical fibroxanthoma and pleomorphic lipoma, they are aggressive neoplasms and considered high grade for management purposes. The most commonly reported pleomorphic sarcoma is pleomorphic MFH, also known as undifferentiated high-grade pleomorphic sarcoma. With wide sampling and immunoprofiling, most “first-impression” pleomorphic MFHs prove to be other poorly differentiated malignant neoplasms (e.g., pleomorphic osteosarcomas, leiomyosarcomas, RMSs, liposarcomas, or high-grade myxofibrosarcomas). In those instances when all efforts fail to reveal a line of differentiation, undifferentiated high-grade pleomorphic sarcoma is preferable to the histogenesis-implicating term *malignant fibrous histiocyoma*.^{9,68,157-159}

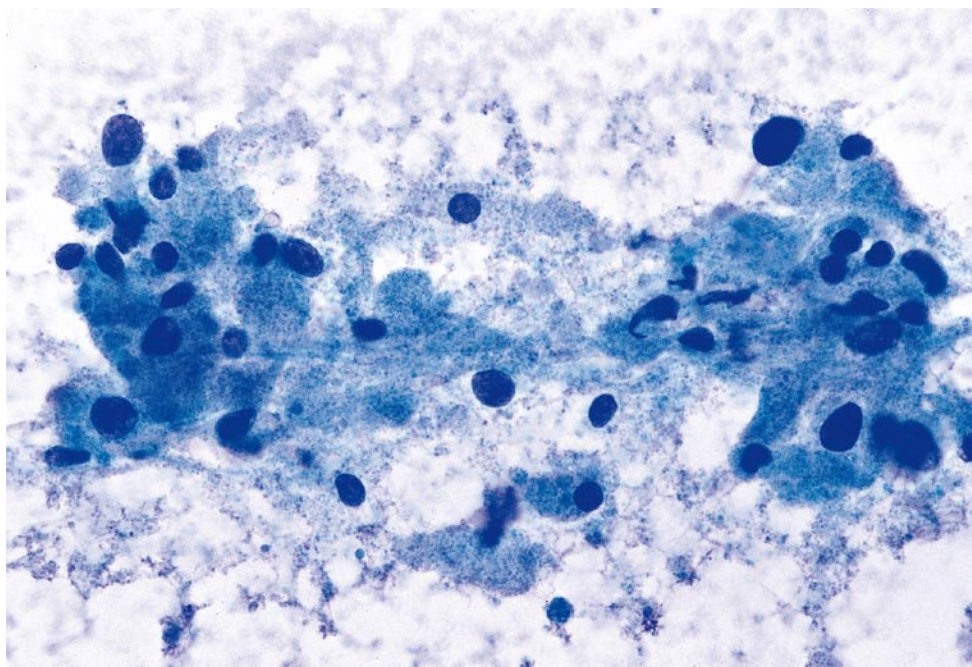


Figure 16.33 Granular cell tumor. Small nuclei within abundant cytoplasm result in a low nuclear-to-cytoplasmic ratio (Papanicolaou stain).

Undifferentiated High-Grade Pleomorphic Sarcoma (Pleomorphic Malignant Fibrous Histiocytoma)

Undifferentiated high-grade pleomorphic sarcoma represents the most common types of sarcomas in patients over age 40 with a peak incidence in the sixth and seventh decades. Most tumors present as a rapidly growing mass (> 5cm) in the deep soft tissue of extremities, especially the lower limb.¹⁵⁹

CYTMORPHOLOGY OF UNDIFFERENTIATED HIGH-GRADE PLEOMORPHIC SARCOMA:

- hypercellular smears
- variable proportions of cellular clusters and dispersed cells
- marked cellular and nuclear pleomorphism and anaplasia
- giant cells with solitary or multiple nuclei
- numerous mitoses including atypical forms
- necrosis

The highly cellular smears contain variable proportions of cellular clusters and dispersed cells in a necrotic background. All the tumors in this group share impressive morphologic features that include markedly pleomorphic, spindle-shaped or epithelioid cells, polygonal cells with abundant cytoplasm, giant cells with soli-

tary or multiple nuclei, prominent necrosis, and brisk mitotic activity with atypical forms (Fig. 16.34). It is difficult to identify any cytomorphologic clues to suggest a line of differentiation in these tumors. The tumor cells can be focally positive for SMA or vimentin, but they are usually negative for all other specific markers by immunohistochemical studies. Cytogenetics usually reveals highly complex karyotypes with marked intratumoral heterogeneity.^{1,69,159}

DIFFERENTIAL DIAGNOSIS OF UNDIFFERENTIATED HIGH-GRADE PLEOMORPHIC SARCOMA

- sarcomatoid carcinoma
- sarcomatoid mesothelioma
- melanoma
- anaplastic large cell lymphoma
- pleomorphic sarcoma with a specific line of differentiation (e.g., RMS, liposarcoma)
- dedifferentiated sarcoma

Sarcomatoid carcinoma, sarcomatoid mesothelioma, malignant melanoma, and anaplastic large cell lymphoma (ALCL) can have the same pleomorphism, including a mixture of highly atypical spindle cells, epithelioid cells, and giant cells. Immunohistochemical studies are usually necessary to make the distinction. Sarcomatoid carcinoma and mesothelioma should have at least focal positivity for one or more cytokeratins, and mesothelioma is additionally positive for mesothelial cell markers

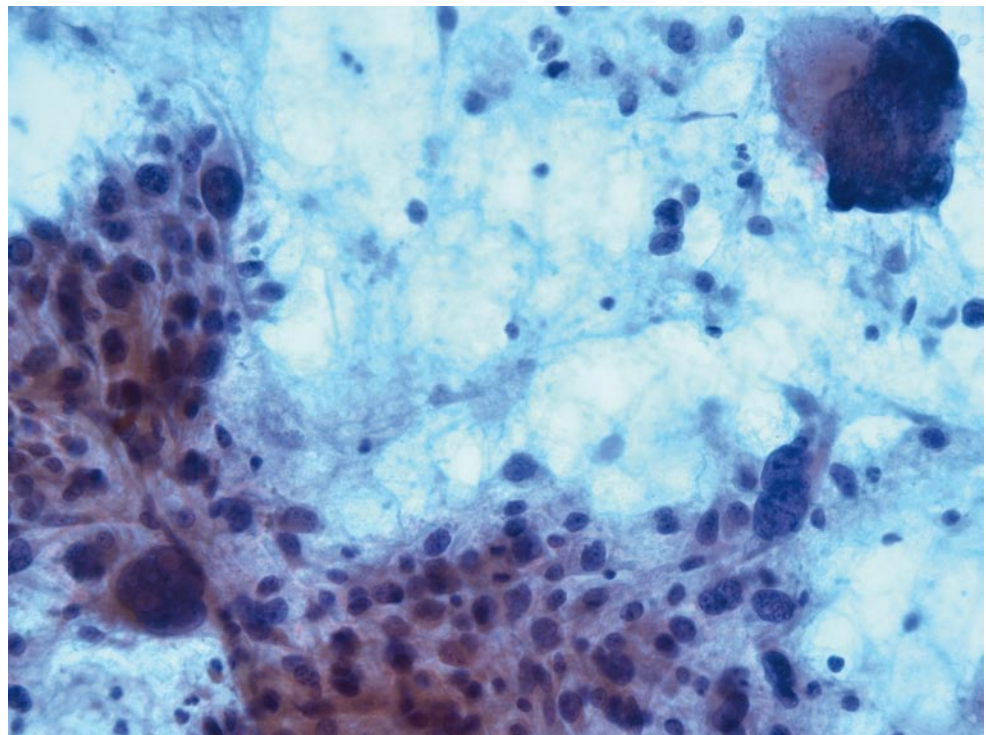


Figure 16.34 Undifferentiated high-grade pleomorphic sarcoma. The bizarre tumor cells and necrotic background are nonspecific findings seen with any pleomorphic soft tissue neoplasm (Papanicolaou stain).

(calretinin, WT1, and D2-40). ALCL shows discohesive large to anaplastic rounded cells with an embryo-like nucleus (see Fig. 11.26). ALCL cells are positive for CD30 and in some cases for ALK as well. Melanoma often has melanin pigment deposition in tumor cells and in adjacent macrophages, and the diagnosis can be confirmed by immunoreactivity for melanocytic markers. A differentiated pleomorphic sarcoma, like pleomorphic liposarcoma or pleomorphic RMS (see Fig. 4.34) is confirmed by demonstrating a clear line of differentiation with ancillary techniques. Clinical history and the presence of a differentiated area are essential for the diagnosis of a dedifferentiated sarcoma. Finally, the diagnosis of a radiation-induced high-grade sarcoma should be considered when an undifferentiated high-grade pleomorphic sarcoma occurs at the site of prior radiation therapy.

Because an FNA represents only a portion of the neoplasm, excluding carcinoma, mesothelioma, melanoma and lymphoma might not be possible because of sampling error. A pleomorphic neoplasm that is negative for differentiation markers might still be best reported as “Pleomorphic neoplasm, cannot classify further.”

Dedifferentiated Sarcomas

Three different soft tissue tumors can undergo dedifferentiation: *well-differentiated liposarcoma*, *well-differentiated chondrosarcoma*, and *chordoma*. Recent rapid growth of a previously stable mass is typical of all three tumors, and the correct classification of each hinges on identifying an adjacent well-differentiated component. For this reason, cytologists must boldly request additional, wider sampling when confronted with an undifferentiated pleomorphic or giant cell lesion during rapid evaluation.

Dedifferentiation occurs with approximately 10% of all chondrosarcomas, usually in patients about 10 years older than those with a conventional chondrosarcoma. Dedifferentiated chordoma is an exceedingly rare event.

Dedifferentiated liposarcoma is the most common of the three and accounts for up to 10% of liposarcomas. It is usually retroperitoneal or intra-abdominal. Its morphology is variable, but most cases resemble either an undifferentiated pleomorphic sarcoma or a myxofibrosarcoma (see Fig. 16.15). Although the transition between the dedifferentiated and well-differentiated components usually occurs in an abrupt fashion, in rare cases it can be a gradual intermingling mimicking pleomorphic liposarcoma in limited samples. In exceptional cases, rare lipoblasts are present in otherwise nonlipogenic areas. Cytogenetic analysis can assist with the cytologic diagnosis because dedifferentiated liposarcoma, in addition to complex aberrations, retains the same giant marker and ring chromosomes as its well-differentiated counterpart.¹⁶⁰⁻¹⁶² Detection of *MDM2* and *CDK4* gene amplifications, which result from these chromosomal

alterations, by molecular cytogenetic approaches (FISH or PCR) can be of great diagnostic value when a routine karyotype cannot be obtained.^{44,163}

NON-NEOPLASTIC SOFT TISSUE LESIONS

Idiopathic Retroperitoneal Fibrosis

Idiopathic retroperitoneal fibrosis is an enigmatic lesion affecting mostly males in their fourth to fifth decades of life. The typical lesion shows bilateral periureteral fibrosis at the level of the L4 vertebra, often encasing the aorta.¹⁶⁴

CYTOMORPHOLOGY OF IDIOPATHIC RETROPERITONEAL FIBROSIS:

- mixed inflammatory infiltrate
- prominent crush artifact
- fragments of fibrous tissue
- spindle-shaped fibroblastic cells

The moderately cellular smears contain rare fragments of fibrous tissue and inflammatory cells including small lymphocytes, plasma cells, histiocytes, rare eosinophils, and mast cells. Neutrophils are also occasionally present. The inflammatory cells are partially obscured by prominent crush artifact. Sparse, benign-appearing, spindle-shaped fibroblastic cells are visible in thicker fragments of the fibrous tissue. Nuclei lack atypia and are cigar-shaped, euchromatic, and have distinct, uniform borders. Nucleoli are inconspicuous, and mitoses and necrosis are absent. The inflammatory infiltrate has a slight predominance of B cells over T cells (60 versus 40%).^{165,166}

DIFFERENTIAL DIAGNOSIS OF IDIOPATHIC RETROPERITONEAL FIBROSIS:

- Hodgkin disease
- non-Hodgkin lymphoma
- well-differentiated inflammatory liposarcoma
- metastatic carcinoma with desmoplastic stroma
- sclerosing mesenteritis

The differential diagnosis includes any lesion with prominent fibrosis and inflammation. The nodular sclerosis variant of Hodgkin lymphoma has distinguishing Reed-Sternberg cells, lacunar cells, and associated clinical features. The lymphoid component of non-Hodgkin lymphoma is usually more monomorphic. Well-differentiated inflammatory liposarcoma should have lipoblasts and more cellular atypia. The desmoplastic stroma of a metastatic carcinoma can resemble the fibrotic areas of idiopathic retroperitoneal fibrosis, and thus immunocytochemistry for keratins and EMA

are helpful in excluding this possibility. Sclerosing mesenteritis usually involves the small bowel mesentery, with more prominent fat necrosis.¹⁶⁴⁻¹⁶⁶

Nodular Fasciitis

Nodular fasciitis is a relatively common fibrous proliferation that typically occurs in subcutaneous tissue. It occurs in all age groups but is most common in young adults. The most common sites are the upper extremities, trunk, and head and neck (including salivary gland), but any part of the body can be affected. It has a typical clinical presentation: rapid growth over a relatively short period of time, usually no more than 2 months. The lesion usually measures less than 2 cm in greatest dimension and is rarely bigger than 5 cm. It can be sore or tender. Only a minority of patients report a history of trauma to the affected area. Because of recent cytogenetic demonstration of clonality, the traditional view of nodular fasciitis as a non-neoplastic, reactive lesion has been challenged.

CYTOMORPHOLOGY OF NODULAR FASCIITIS:

- myxoid background
- numerous dispersed polymorphic (e.g., spindle-shaped, stellate) myofibroblasts
- lack of significant hyperchromasia
- uninucleated or binucleated ganglion-like cells with eccentric nuclei and prominent nucleoli
- inflammatory cells

The moderately hypercellular aspirates have a heterogeneous background of myxoid material, inflammatory

cells, and delicate branching vessels. Variably cellular fragments of collagen are occasionally present. There are numerous solitary spindle-shaped and stellate myofibroblastic cells (Fig. 16.35), but some also form cohesive groups. The presence of uninucleated or binucleated ganglion-like cells is a characteristic finding. Nuclei are uniform and lack significant atypia. They are small in the fusiform cells and somewhat larger in the plumper stellate cells. Their chromatin is delicate and open, and the inapparent nucleolus of the spindle-shaped cells is more distinct in the larger cells. Cytoplasm is generally abundant, but wispy with tapering ends and ill-defined borders. Mitotic figures can be alarmingly numerous. The myofibroblasts are positive for smooth muscle actin and pan-muscle actin, but negative for desmin and S-100 protein, compatible with their myofibroblastic nature.¹⁶⁷⁻¹⁷⁰

Nodular fasciitis is one of the most notorious mimics of malignancy among all the soft tissue lesions. The differential diagnosis includes a variety of sarcomas. The key to arriving at the correct (benign!) diagnosis is the correlation of clinical information with the FNA findings. The diagnosis is often suggested just by the clinical presentation (e.g., a rapidly growing subcutaneous mass smaller than 2 cm), and the typical FNA findings serve simply to confirm this clinical suspicion.

Elastofibroma

Elastofibroma is a relatively uncommon lesion thought to represent a reactive process, possibly from repeated trauma to the tissues between the scapulae and the chest wall. Elderly individuals, particularly women, are most commonly affected. It presents as a slowly growing subscapular mass attached to the rib periosteum.

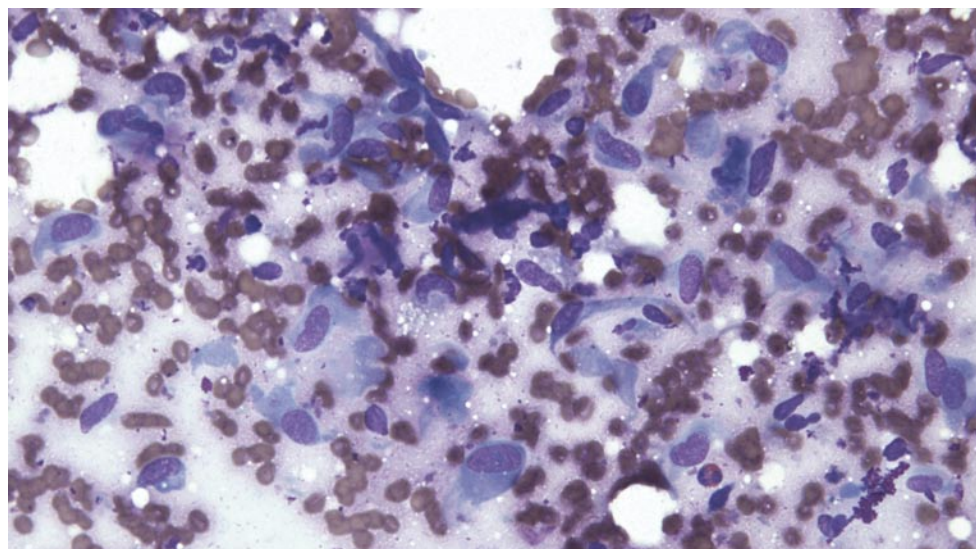


Figure 16.35 Nodular fasciitis. Numerous dispersed, polymorphic myofibroblasts are characteristic (Romanowsky stain).

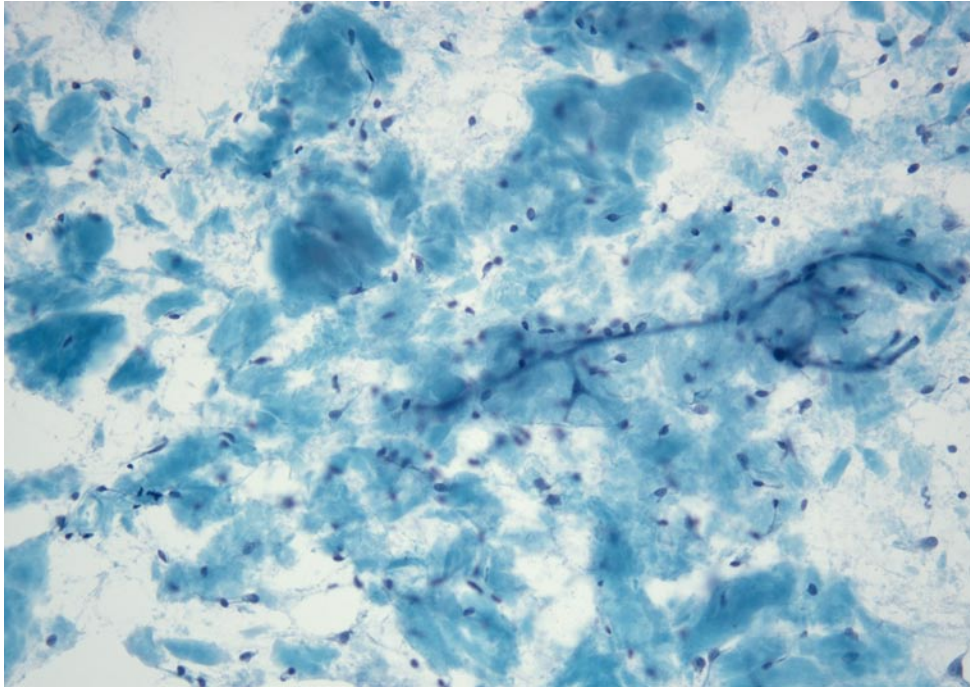


Figure 16.36 Amyloidoma. Fragments of waxy, amorphous material have sharply defined edges with embedded fibroblasts and capillaries (Papanicolaou stain).

CYTOMORPHOLOGY OF ELASTOFIBROMA:

- hypocellular, waxy matrix
- linear, dentate or serrated, rodlike structures
- serrated globular bodies
- scant uniform bland fibroblasts

Cytologic preparations are remarkably hypocellular. Rare fibroblasts with bland nuclear features are seen within a homogeneous, waxy, hyalinized matrix. These stromal fragments have sharp borders. Linear, slightly curved or angulated, rodlike elastic fibers of varying sizes, lengths, and widths typically have dentations or serrations along the long edge and should not be mistaken for degenerated fungi. The globular bodies also have serrated borders and vary in diameter. Most are solitary, but occasional loose or tight clusters are found. A nonspecific diagnosis is avoided only if the elastic fibers and globular bodies are noted.^{171,172}

Amyloidoma (Tumoral Amyloidosis)

An amyloidoma is an uncommon mass lesion caused by extensive amyloid deposition. Some but not all cases are associated with systemic amyloidosis. It can occur in varied visceral organs, and extremely rarely in bone and soft tissues.

CYTOMORPHOLOGY OF AMYLOIDOMA:

- fragments of waxy, amorphous material with sharply defined edges and embedded fibroblasts and capillaries
- multinucleated giant cells
- scant inflammatory cells including plasma cells

Cytologic preparations are hypocellular and contain abundant fragments of acellular, waxy, amorphous material with sharply defined edges and bland mesenchymal cells. (Fig. 16.36). It is a metachromatic blue-purple with Romanowsky stains and pink, orange, or green with the Papanicolaou stain. Scattered multinucleated giant cells are common. Inflammatory cells including plasma cells may or may not be present. A nonspecific diagnosis is avoided only if the amyloid deposition is confirmed by a Congo red stain or ultrastructural analysis.^{173,174}

REFERENCES

1. Akerman M, Domanski HA: The cytology of soft tissue tumours. *Monogr Clin Cytol* 2003;16:IX-X, 1-112.
2. Akerman M, Willen H: Critical review on the role of fine needle aspiration in soft tissue tumors. *Path Case Rev* 1998; 3(3):111-117.
3. Jemal A, Siegel R, Ward E, Murray T, et al.: Cancer statistics, 2007. *CA Cancer J Clin* 2007;57(1):43-66.
4. Kilpatrick SE, Geisinger KR: Soft tissue sarcomas: The usefulness and limitations of fine-needle aspiration biopsy. *Am J Clin Pathol* 1998;110(1):50-68.
5. Bennert KW, Abdul-Karim FW: Fine needle aspiration cytology vs. needle core biopsy of soft tissue tumors. A comparison. *Acta Cytol* 1994;38(3):381-384.
6. Gonzalez-Campora R: Fine needle aspiration cytology of soft tissue tumors. *Acta Cytol* 2000;44(3):337-343.
7. Liu K, Layfield LJ, Coogan AC, et al.: Diagnostic accuracy in fine-needle aspiration of soft tissue and bone lesions. Influence of clinical history and experience. *Am J Clin Pathol* 1999;111(5):632-640.
8. Kilpatrick SE, Cappellari JO, Bos GD, et al.: Is fine-needle aspiration biopsy a practical alternative to open biopsy for the primary diagnosis of sarcoma? Experience with 140 patients. *Am J Clin Pathol* 2001;115(1):59-68.

9. Kilpatrick SE, Ward WG, Cappellari JO, Bos GD: Fine-needle aspiration biopsy of soft tissue sarcomas. A cytomorphologic analysis with emphasis on histologic subtyping, grading, and therapeutic significance. *Am J Clin Pathol* 1999;112(2):179-88.
10. Kilpatrick SE, Ward WG, Chauvenet AR, Pettenati MJ: The role of fine-needle aspiration biopsy in the initial diagnosis of pediatric bone and soft tissue tumors: An institutional experience. *Mod Pathol* 1998;11(10):923-928.
11. Nemanqani D, Mourad WA: Cytomorphologic features of fine-needle aspiration of liposarcoma. *Diagn Cytopathol* 1999;20(2):67-69.
12. Skoog L, Pereira ST, Tani E: Fine-needle aspiration cytology and immunocytochemistry of soft-tissue tumors and osteo/chondrosarcomas of the head and neck. *Diagn Cytopathol* 1999;20(3):131-136.
13. Antonescu CR: The role of genetic testing in soft tissue sarcoma. *Histopathology* 2006;48(1):13-21.
14. Ferretti M, Gusella PM, Mancini AM, et al.: Progressive approach to the cytologic diagnosis of retroperitoneal spindle cell tumors. *Acta Cytol* 1997;41(2):450-460.
15. Logrono R, Wojtowycz MM, Wunderlich DW, et al.: Fine needle aspiration cytology and core biopsy in the diagnosis of alveolar soft part sarcoma presenting with lung metastases. A case report. *Acta Cytol* 1999;43(3):464-470.
16. Powers CN, Berardo MD, Frable WJ: Fine-needle aspiration biopsy: Pitfalls in the diagnosis of spindle-cell lesions. *Diagn Cytopathol* 1994;10(3):232-240; discussion 241.
17. Dey P, Mallik MK, Gupta SK, Vasishta RK: Role of fine needle aspiration cytology in the diagnosis of soft tissue tumours and tumour-like lesions. *Cytopathology* 2004;15(1):32-37.
18. Costa MJ, Campman SC, Davis RL, Howell LP: Fine-needle aspiration cytology of sarcoma: Retrospective review of diagnostic utility and specificity. *Diagn Cytopathol* 1996;15(1):23-32.
19. Palmer HE, Mukunyadzi P, Culbreth W, Thomas JR: Subgrouping and grading of soft-tissue sarcomas by fine-needle aspiration cytology: A histopathologic correlation study. *Diagn Cytopathol* 2001;24(5):307-316.
20. Yu GH, Sack MJ, Baloch Z, Gupta PK: Difficulties in the fine needle aspiration (FNA) diagnosis of schwannoma. *Cytopathology* 1999;10(3):186-194.
21. Gonzalez-Campora R: Cytoarchitectural findings in the diagnosis of primary soft tissue tumors. *Acta Cytol* 2001;45(2):115-146.
22. Wakely PE Jr, Geisinger KR, Cappellari JO, et al.: Fine-needle aspiration cytopathology of soft tissue: Chondromyxoid and myxoid lesions. *Diagn Cytopathol* 1995;12(2):101-105.
23. Thunnissen FB, Kroese AH, Ambergen AW, et al.: Which cytological criteria are the most discriminative to distinguish carcinoma, lymphoma, and soft-tissue sarcoma? A probabilistic approach. *Diagn Cytopathol* 1997;17(5):333-338.
24. Akerman M, Rydholm A: Aspiration cytology of lipomatous tumors: A 10-year experience at an orthopedic oncology center. *Diagn Cytopathol* 1987;3(4):295-302.
25. Guter GE, Gatscha RM, Zakowski MF: ThinPrep vs. conventional smears in fine-needle aspirations of sarcomas: A morphological and immunocytochemical study. *Diagn Cytopathol* 1999;21(5):351-354.
26. Michael CW, Hunter B: Interpretation of fine-needle aspirates processed by the ThinPrep technique: Cytologic artifacts and diagnostic pitfalls. *Diagn Cytopathol* 2000;23(1):6-13.
27. Tong TR, Chow TC, Chan OW, et al.: Clear-cell sarcoma diagnosis by fine-needle aspiration: Cytologic, histologic, and ultrastructural features; potential pitfalls; and literature review. *Diagn Cytopathol* 2002;26(3):174-180.
28. Kindblom LG, Walaas L, Widehn S: Ultrastructural studies in the preoperative cytologic diagnosis of soft tissue tumors. *Semin Diagn Pathol* 1986;3(4):317-344.
29. McGahey BE, Moriarty AT, Nelson WA, Hull MT: Fine-needle aspiration biopsy of small round blue cell tumors of childhood. *Cancer* 1992;69(4):1067-1073.
30. Silverman JF, Joshi VV: FNA biopsy of small round cell tumors of childhood: Cytomorphologic features and the role of ancillary studies. *Diagn Cytopathol* 1994;10(3):245-255.
31. Sharma M, Ahsan F, Ah-See KW, et al.: Interdigitating dendritic cell sarcoma of the parotid gland. *J Laryngol Otol* 2006;120(3):244-246.
32. Akhtar M, Iqbal MA, Mourad W, Ali MA: Fine-needle aspiration biopsy diagnosis of small round cell tumors of childhood: A comprehensive approach. *Diagn Cytopathol* 1999;21(2):81-91.
33. Pohar-Marinsek Z, Srebotnik-Kirbis I: Desmin detection in FNAB samples of rhabdomyosarcoma: An immunocytochemical study. *Cytopathology* 2000;11(3):171-178.
34. Molenaar WM, van den Berg E, Dolfin AC, et al.: Cytogenetics of fine needle aspiration biopsies of sarcomas. *Cancer Genet Cytogenet* 1995;84(1):27-31.
35. Saboorian MH, Ashfaq R, Vandersteenhoven JJ, Schneider NR: Cytogenetics as an adjunct in establishing a definitive diagnosis of synovial sarcoma by fine-needle aspiration. *Cancer* 1997;81(3):187-192.
36. Akerman M, Dreinhofer K, Rydholm A, et al.: Cytogenetic studies on fine-needle aspiration samples from osteosarcoma and Ewing's sarcoma. *Diagn Cytopathol* 1996;15(1):17-22.
37. Abati A, Sanford JS, Fetsch P, et al.: Fluorescence in situ hybridization (FISH): A user's guide to optimal preparation of cytologic specimens. *Diagn Cytopathol* 1995;13(5):486-492.
38. Recommendations for the reporting of soft tissue sarcomas. Association of Directors of Anatomic and Surgical Pathology. *Mod Pathol* 1998;11(12):1257-1261.
39. Brown FM, Fletcher CD: Problems in grading soft tissue sarcomas. *Am J Clin Pathol* 2000;114 Suppl:S82-S89.
40. Weir MM, Rosenberg AE, Bell DA: Grading of spindle cell sarcomas in fine-needle aspiration biopsy specimens. *Am J Clin Pathol* 1999;112(6):784-790.
41. Kapila K, Ghosal N, Gill SS, Verma K: Cytomorphology of lipomatous tumors of soft tissue. *Acta Cytol* 2003;47(4):555-562.
42. Gonzalez-Campora R, Munoz-Arias G, Otal-Salaverri C, et al.: Fine needle aspiration cytology of primary soft tissue tumors. Morphologic analysis of the most frequent types. *Acta Cytol* 1992;36(6):905-917.
43. Walaas L, Kindblom LG: Lipomatous tumors: A correlative cytologic and histologic study of 27 tumors examined by fine needle aspiration cytology. *Hum Pathol* 1985;16(1):6-18.
44. Fletcher CDM (ed): *Diagnostic Histopathology of Tumors*, 3rd ed. London, Churchill Livingstone, 2007.
45. Lemos MM, Kindblom LG, Meis-Kindblom JM, et al.: Fine-needle aspiration characteristics of hibernoma. *Cancer* 2001;93(3):206-210.
46. Guo Z, Voytovich M, Kurtycz DF, Hoerl HD: Fine-needle aspiration diagnosis of spindle-cell lipoma: A case report and review of the literature. *Diagn Cytopathol* 2000;23(5):362-365.
47. Domanski HA, Carlen B, Jonsson K, et al.: Distinct cytologic features of spindle cell lipoma. A cytologic-histologic study with clinical, radiologic, electron microscopic, and cytogenetic correlations. *Cancer* 2001;93(6):381-389.
48. Lopez-Rios F, Alberti N, Perez-Barrios A, de Agustin PP: Fine-needle aspiration of pleomorphic lipoma. *Diagn Cytopathol* 2001;24(4):296-297.
49. Rubin BP, Dal Cin P: The genetics of lipomatous tumors. *Semin Diagn Pathol* 2001;18(4):286-293.

50. Dey P: Fine needle aspiration cytology of well-differentiated liposarcoma. A report of two cases. *Acta Cytol* 2000;44(3):459-462.
51. Woyke S, Kapila K, Goswami KC: Atypical lipoma as a potential pitfall in the cytodiagnosis of subcutaneous tumors. A report of two cases. *Acta Cytol* 1997;41(3):897-902.
52. Wu HH, Harshbarger KE, McCandless DG: Fine-needle aspiration cytology of well-differentiated inflammatory liposarcoma: A case report with histologic follow-up. *Diagn Cytopathol* 1999;20(4):229-232.
53. Kljaniienko J, Caillaud JM, Lagace R: Fine-needle aspiration in liposarcoma: Cytohistologic correlative study including well-differentiated, myxoid, and pleomorphic variants. *Diagn Cytopathol* 2004;30(5):307-312.
54. Romero-Guadarrama MB, Jimenez-Becerra S, Duran-Padilla MA, et al.: Mediastinal pleomorphic liposarcoma diagnosed by fine needle aspiration biopsy: A case report. *Acta Cytol* 2007;51(3):440-442.
55. Fletcher CDM, Unni KK, Mertens F (eds): World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. Lyon, France, IARC Press, 2002.
56. Gonzalez-Campora R, Otal-Salaverri C, Hevia-Vazquez A, et al.: Fine needle aspiration in myxoid tumors of the soft tissues. *Acta Cytol* 1990;34(2):179-191.
57. Wakely PE Jr, Bos GD, Mayerson J: The cytopathology of soft tissue myxomas: Ganglia, juxta-articular myxoid lesions, and intramuscular myxoma. *Am J Clin Pathol* 2005;123(6):858-865.
58. Caraway NP, Staerckel GA, Fanning CV, et al.: Diagnosing intramuscular myxoma by fine-needle aspiration: A multidisciplinary approach. *Diagn Cytopathol* 1994;11(3):255-261.
59. Kilpatrick SE, Ward WG: Myxofibrosarcoma of soft tissues: cytomorphologic analysis of a series. *Diagn Cytopathol* 1999;20(1):6-9.
60. Kilpatrick SE, Ward WG, Bos GD: The value of fine-needle aspiration biopsy in the differential diagnosis of adult myxoid sarcoma. *Cancer* 2000;90(3):167-177.
61. Layfield LJ, Liu K, Dodge RK: Logistic regression analysis of myxoid sarcomas: A cytologic study. *Diagn Cytopathol* 1998;19(5):355-360.
62. Evans HL: Low-grade fibromyxoid sarcoma. A report of two metastasizing neoplasms having a deceptively benign appearance. *Am J Clin Pathol* 1987;88(5):615-619.
63. Lindberg GM, Maitra A, Gokaslan ST, et al.: Low grade fibromyxoid sarcoma: Fine-needle aspiration cytology with histologic, cytogenetic, immunohistochemical, and ultrastructural correlation. *Cancer* 1999;87(2):75-82.
64. Silverman JF, Nathan G, Olson PR, et al.: Fine-needle aspiration cytology of low-grade fibromyxoid sarcoma of the renal capsule (capsuloma). *Diagn Cytopathol* 2000;23(4):279-283.
65. Dawamneh ME, Amra NK, Amr SS: Low grade fibromyxoid sarcoma: Report of a case with fine needle aspiration cytology and histologic correlation. *Acta Cytol* 2006;50(2):208-212.
66. Mertens F, Fletcher CD, Antonescu CR, et al.: Clinicopathologic and molecular genetic characterization of low-grade fibromyxoid sarcoma, and cloning of a novel FUS/CREB3L1 fusion gene. *Lab Invest* 2005;85(3):408-415.
67. Reid R, de Silva MV, Paterson L, et al.: Low-grade fibromyxoid sarcoma and hyalinizing spindle cell tumor with giant rosettes share a common t(7;16)(q34;p11) translocation. *Am J Surg Pathol* 2003;27(9):1229-1236.
- 67a. Evans HL: Liposarcoma: a study of 55 cases with a reassessment of its classification. *Am J Surg Pathol* 1979;3(6):507-523.
68. Fletcher CD, Gustafson P, Rydholm A, et al.: Clinicopathologic re-evaluation of 100 malignant fibrous histiocytomas: Prognostic relevance of subclassification. *J Clin Oncol* 2001;19(12):3045-3050.
69. Kljaniienko J, Caillaud JM, Lagace R, Vielh P: Comparative fine-needle aspiration and pathologic study of malignant fibrous histiocytoma: Cytohistologic features of 95 tumors in 71 patients. *Diagn Cytopathol* 2003;29(6):320-326.
70. Kloboves-Prevodnik VV, Us-Krasovec M, Gale N, Lamovec J: Cytological features of lipoblastoma: A report of three cases. *Diagn Cytopathol* 2005;33(3):195-200.
71. Lopez-Ferrer P, Jimenez-Heffernan JA, Yebenes L, et al.: Fine-needle aspiration cytology of lipoblastoma: A report of two cases. *Diagn Cytopathol* 2005;32(1):32-34.
72. Kay PA, Nascimento AG, Unni KK, Salomao DR: Chordoma. Cytomorphologic findings in 14 cases diagnosed by fine needle aspiration. *Acta Cytol* 2003;47(2):202-208.
73. Layfield LJ: Cytologic differential diagnosis of myxoid and mucinous neoplasms of the sacrum and parasacral soft tissues. *Diagn Cytopathol* 2003;28(5):264-271.
74. Crapanzano JP, Ali SZ, Ginsberg MS, Zakowski MF: Chordoma: A cytologic study with histologic and radiologic correlation. *Cancer* 2001;93(1):40-51.
75. Niemann TH, Bottles K, Cohen MB: Extraskeletal myxoid chondrosarcoma: Fine-needle aspiration biopsy findings. *Diagn Cytopathol* 1994;11(4):363-366.
76. Orndal C, Carlen B, Akerman M, et al.: Chromosomal abnormality t(9;22)(q22;q12) in an extraskeletal myxoid chondrosarcoma characterized by fine needle aspiration cytology, electron microscopy, immunohistochemistry and DNA flow cytometry. *Cytopathology* 1991;2(5):261-270.
77. Wadhwa N, Arora VK, Singh N, Bhatia A: Fine needle aspiration cytology of primary extraskeletal myxoid chondrosarcoma. A case report. *Acta Cytol* 2000;44(3):445-448.
78. Jakowski JD, Wakely PE Jr: Cytopathology of extraskeletal myxoid chondrosarcoma: Report of 8 cases. *Cancer* 2007;111(5):298-305.
79. Domanski HA, Akerman M, Rissler P, Gustafson P: Fine-needle aspiration of soft tissue leiomyosarcoma: An analysis of the most common cytologic findings and the value of ancillary techniques. *Diagn Cytopathol* 2006;34(9):597-604.
80. Kljaniienko J, Caillaud JM, Lagace R, Vielh P: Fine-needle aspiration of leiomyosarcoma: A correlative cytohistopathological study of 96 tumors in 68 patients. *Diagn Cytopathol* 2003;28(3):119-125.
81. Tao LC, Davidson DD: Aspiration biopsy cytology of smooth muscle tumors. A cytologic approach to the differentiation between leiomyosarcoma and leiomyoma. *Acta Cytol* 1993;37(3):300-308.
82. Domanski HA, Akerman M, Engellau J, et al.: Fine-needle aspiration of neurilemoma (schwannoma). A clinicocytopathologic study of 116 patients. *Diagn Cytopathol* 2006;34(6):403-412.
83. Mooney EE, Layfield LJ, Dodd LG: Fine-needle aspiration of neural lesions. *Diagn Cytopathol* 1999;20(1):1-5.
84. Kljaniienko J, Caillaud JM, Lagace R: Cytohistologic correlations in schwannomas (neurilemmomas), including "ancient," cellular, and epithelioid variants. *Diagn Cytopathol* 2006;34(8):517-522.
85. Gupta K, Dey P, Vashisht R: Fine-needle aspiration cytology of malignant peripheral nerve sheath tumors. *Diagn Cytopathol* 2004;31(1):1-4.
86. McGee RS Jr, Ward WG, Kilpatrick SE: Malignant peripheral nerve sheath tumor: a fine-needle aspiration biopsy study. *Diagn Cytopathol* 1997;17(4):298-305.
87. Kljaniienko J, Caillaud JM, Lagace R, Vielh P: Cytohistologic correlations of 24 malignant peripheral nerve sheath tumor (MPNST) in 17 patients: The Institut Curie experience. *Diagn Cytopathol* 2002;27(2):103-108.
88. Inagaki H, Murase T, Otsuka T, Eimoto T: Detection of SYT-SSX fusion transcript in synovial sarcoma using archival cytologic specimens. *Am J Clin Pathol* 1999;111(4):528-533.

89. Nilsson G, Wang M, Wejde J, et al. Reverse transcriptase polymerase chain reaction on fine needle aspirates for rapid detection of translocations in synovial sarcoma. *Acta Cytol* 1998;42(6):1317-1324.
90. Kljanienco J, Caillaud JM, Lagace R, Vielh P: Cytohistologic correlations in 56 synovial sarcomas in 36 patients: The Institut Curie experience. *Diagn Cytopathol* 2002;27(2):96-102.
91. Kilpatrick SE, Teot LA, Stanley MW, et al.: Fine-needle aspiration biopsy of synovial sarcoma. A cytomorphologic analysis of primary, recurrent, and metastatic tumors. *Am J Clin Pathol* 1996;106(6):769-775.
92. Ryan MR, Stastny JF, Wakely PE Jr: The cytopathology of synovial sarcoma: A study of six cases, with emphasis on architecture and histopathologic correlation. *Cancer* 1998;84(1):42-9.
93. Akerman M, Ryd W, Skytting B: Fine-needle aspiration of synovial sarcoma: Criteria for diagnosis. Retrospective reexamination of 37 cases, including ancillary diagnostics. A Scandinavian Sarcoma Group study. *Diagn Cytopathol* 2003;28(5):232-238.
94. Akerman M, Domanski HA: The complex cytological features of synovial sarcoma in fine needle aspirates, an analysis of four illustrative cases. *Cytopathology* 2007;18(4):234-240.
95. Gengler C, Guillou L: Solitary fibrous tumour and haemangiopericytoma: Evolution of a concept. *Histopathology* 2006; 48(1):63-74.
96. Clayton AC, Salomao DR, Keeney GL, Nascimento AG: Solitary fibrous tumor: A study of cytologic features of six cases diagnosed by fine-needle aspiration. *Diagn Cytopathol* 2001;25(3):172-176.
97. Ali SZ, Hoon V, Hoda S, et al.: Solitary fibrous tumor. A cytologic-histologic study with clinical, radiologic, and immunohistochemical correlations. *Cancer* 1997;81(2):116-121.
98. Cho EY, Han JJ, Han J, Oh YL: Fine needle aspiration cytology of solitary fibrous tumours of the pleura. *Cytopathology* 2007;18(1):20-27.
99. Domanski HA: Fine-needle aspiration smears from lipomatous hemangiopericytoma need not be confused with myxoid liposarcoma. *Diagn Cytopathol* 2003;29(5):287-291.
100. Owens CL, Sharma R, Ali SZ: Deep fibromatosis (desmoid tumor): Cytopathologic characteristics, clinicoradiologic features, and immunohistochemical findings on fine-needle aspiration. *Cancer* 2007;111(3):166-172.
101. Raab SS, Silverman JF, McLeod DL, et al.: Fine needle aspiration biopsy of fibromatoses. *Acta Cytol* 1993;37(3):323-328.
102. Powers CN, Hurt MA, Frable WJ: Fine-needle aspiration biopsy: Dermatofibrosarcoma protuberans. *Diagn Cytopathol* 1993;9(2):145-150.
103. Domanski HA, Gustafson P: Cytologic features of primary, recurrent, and metastatic dermatofibrosarcoma protuberans. *Cancer* 2002;96(6):351-361.
104. Hannah CD, Oliver DH, Liu J: Fine needle aspiration biopsy and immunostaining findings in an aggressive inflammatory myofibroblastic tumor of the lung: a case report. *Acta Cytol* 2007;51(2):239-243.
105. Zardawi IM, Clark D, Williams G: Inflammatory myofibroblastic tumor of the breast. A case report. *Acta Cytol* 2003;47(6):1077-1081.
106. Iyer VK, Kapila K, Verma K: Fine-needle aspiration cytology of giant cell tumor of tendon sheath. *Diagn Cytopathol* 2003;29(2):105-110.
107. Gonzalez-Campora R, Salas Herrero E, Otal-Salaverri CV-RJL, et al.: Diffuse tenosynovial giant cell tumor of soft tissues. Report of a case with cytologic and cytogenetic findings. *Acta Cytol* 1995;39(4):770-776.
108. Layfield LJ: *Cytopathology of bone and soft tissue tumors*. New York, Oxford University Press, 2002.
109. Basu S, Singhal U, Mohan H: Fine needle aspiration of angiomatoid malignant fibrous histiocytoma. A case report. *Acta Cytol* 1999;43(5):859-861.
110. Lemos MM, Karlen J, Tani E: Fine-needle aspiration cytology of angiomatoid malignant fibrous histiocytoma. *Diagn Cytopathol* 2005;33(2):116-121.
111. Hallor KH, Micci F, Meis-Kindblom JM, et al.: Fusion genes in angiomatoid fibrous histiocytoma. *Cancer Lett* 2007;251(1):158-163.
112. Guiter GE, Gamboni MM, Zakowski MF: The cytology of extraskeletal Ewing sarcoma. *Cancer* 1999;87(3):141-148.
113. Sahu K, Pai RR, Khadilkar UN: Fine needle aspiration cytology of the Ewing's sarcoma family of tumors. *Acta Cytol* 2000;44(3):332-336.
114. Silverman JF, Berns LA, Holbrook CT, et al.: Fine needle aspiration cytology of primitive neuroectodermal tumors. A report of these cases. *Acta Cytol* 1992;36(4):541-550.
115. Brahmi U, Rajwanshi A, Joshi K, et al.: Role of immunocytochemistry and DNA flow cytometry in the fine-needle aspiration diagnosis of malignant small round-cell tumors. *Diagn Cytopathol* 2001;24(4):233-239.
116. Bakhos R, Andrey J, Bhoopalam N, et al.: Fine-needle aspiration cytology of extraskeletal Ewing's sarcoma. *Diagn Cytopathol* 1998;18(2):137-140.
117. Cohen MC, Pollono D, Tomarchio SA, Drut R: Cytologic characteristics of peripheral neuroectodermal tumors in fine-needle aspiration smears: A retrospective study of three pediatric cases. *Diagn Cytopathol* 1997;16(6):513-517.
118. Sanati S, Lu DW, Schmidt E, et al.: Cytologic diagnosis of Ewing sarcoma/peripheral neuroectodermal tumor with paired prospective molecular genetic analysis. *Cancer* 2007;111(3): 192-199.
119. Renshaw AA, Perez-Atayde AR, Fletcher JA, Granter SR: Cytology of typical and atypical Ewing's sarcoma/PNET. *Am J Clin Pathol* 1996;106(5):620-624.
120. Silverman JF, Landreneau RJ, Sturgis CD, et al.: Small-cell variant of synovial sarcoma: Fine-needle aspiration with ancillary features and potential diagnostic pitfalls. *Diagn Cytopathol* 2000;23(2):118-123.
121. Hummel P, Yang GC, Kumar A, et al.: PNET-like features of synovial sarcoma of the lung: A pitfall in the cytologic diagnosis of soft-tissue tumors. *Diagn Cytopathol* 2001;24(4):283-288.
122. Presley AE, Kong CS, Rowe DM, Atkins KA: Cytology of desmoplastic small round-cell tumor: Comparison of pre- and post-chemotherapy fine-needle aspiration biopsies. *Cancer* 2007;111(1):41-46.
123. Caraway NP, Fanning CV, Amato RJ, et al.: Fine-needle aspiration of intra-abdominal desmoplastic small cell tumor. *Diagn Cytopathol* 1993;9(4):465-470.
124. Crapanzano JP, Cardillo M, Lin O, Zakowski MF: Cytology of desmoplastic small round cell tumor. *Cancer* 2002; 96(1):21-31.
125. Kljanienco J, Caillaud JM, Orbach D, et al.: Cyto-histological correlations in primary, recurrent and metastatic rhabdomyosarcoma: The Institut Curie's experience. *Diagn Cytopathol* 2007;35(8):482-487.
126. Seidal T, Walaas L, Kindblom LG, Angervall L: Cytology of embryonal rhabdomyosarcoma: A cytologic, light microscopic, electron microscopic, and immunohistochemical study of seven cases. *Diagn Cytopathol* 1988;4(4):292-299.
127. Pohar-Marinsek Z, Bracko M: Rhabdomyosarcoma. Cytomorphology, subtyping and differential diagnostic dilemmas. *Acta Cytol* 2000;44(4):524-532.
128. Layfield LJ, Liu K, Dodge RK: Logistic regression analysis of small round cell neoplasms: a cytologic study. *Diagn Cytopathol* 1999;20(5):271-277.

129. Akhtar M, Ali MA, Bakry M, et al.: Fine-needle aspiration biopsy diagnosis of rhabdomyosarcoma: Cytologic, histologic, and ultrastructural correlations. *Diagn Cytopathol* 1992;8(5):465-474.
130. Gonzalez-Campora R, Otal Salaverri C, Gomez Pascual A, et al.: Mesenchymal chondrosarcoma of the retroperitoneum. Report of a case diagnosed by fine needle aspiration biopsy with immunohistochemical, electron microscopic demonstration of S-100 protein in undifferentiated cells. *Acta Cytol* 1995;39(6):1237-1243.
131. Trembath DG, Dash R, Major NM, Dodd LG: Cytopathology of mesenchymal chondrosarcomas: A report and comparison of four patients. *Cancer* 2003;99(4):211-216.
132. Doria MI Jr, Wang HH, Chinoy MJ: Retroperitoneal mesenchymal chondrosarcoma. Report of a case diagnosed by fine needle aspiration cytology. *Acta Cytol* 1990;34(4):529-532.
133. Cardillo M, Zakowski MF, Lin O: Fine-needle aspiration of epithelioid sarcoma: Cytology findings in nine cases. *Cancer* 2001;93(4):246-251.
134. Pohar-Marinsek Z, Zidar A: Epithelioid sarcoma in FNAB smears. *Diagn Cytopathol* 1994;11(4):367-372.
135. Gonzalez-Peramato P, Jimenez-Heffernan JA, Cuevas J: Fine-needle aspiration cytology of "proximal-type" epithelioid sarcoma. *Diagn Cytopathol* 2001;25(2):122-125.
136. Almeida MM, Nunes AM, Frable WJ: Malignant melanoma of soft tissue. A report of three cases with diagnosis by fine needle aspiration cytology. *Acta Cytol* 1994;38(2):241-246.
137. Caraway NP, Fanning CV, Wojcik EM, et al.: Cytology of malignant melanoma of soft parts: Fine-needle aspirates and exfoliative specimens. *Diagn Cytopathol* 1993;9(6):632-638.
138. Maruyama R, Nakano M, Yamashita S, et al.: Fine needle aspiration cytology of clear cell sarcoma. Report of a case with immunocytochemical, immunohistochemical and ultrastructural studies. *Acta Cytol* 1992;36(6):937-942.
139. Lopez-Ferrer P, Jimenez-Heffernan JA, Vicandi B, et al.: Cytologic features of alveolar soft part sarcoma: Report of three cases. *Diagn Cytopathol* 2002;27(2):115-119.
140. Drachenberg CB, Papadimitriou JC: Alveolar soft part sarcoma. A case report with correlation of fine needle aspiration and ultrastructural cytologic features. *Acta Cytol* 1991;35(6):746-752.
141. Shabb N, Sneige N, Fanning CV, Dekmezian R: Fine-needle aspiration cytology of alveolar soft-part sarcoma. *Diagn Cytopathol* 1991;7(3):293-298.
142. Persson S, Willems JS, Kindblom LG, Angervall L: Alveolar soft part sarcoma. An immunohistochemical, cytologic and electron-microscopic study and a quantitative DNA analysis. *Virchows Arch A Pathol Anat Histopathol* 1988;412(6):499-513.
143. Argani P, Lal P, Hutchinson B, et al.: Aberrant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions: a sensitive and specific immunohistochemical assay. *Am J Surg Pathol* 2003;27(6):750-761.
144. Jayaram G, Kapoor R, Saha MM: Hemangioendothelioma. Cytologic appearances in two cases presenting with multiple soft tissue and bone lesions. *Acta Cytol* 1987;31(4):497-501.
145. Kilpatrick SE, Kopley PD, Ward WG, Richards F 2nd: Epithelioid hemangioendothelioma of bone and soft tissue: A fine-needle aspiration biopsy study with histologic and immunohistochemical confirmation. *Diagn Cytopathol* 1998;19(1):38-43.
146. Lin O, Olgac S, Zakowski M: Cytological features of epithelioid mesenchymal neoplasms: A study of 21 cases. *Diagn Cytopathol* 2005;32(1):5-10.
147. Pettinato G, Insabato L, De Chiara A, et al.: Epithelioid heman-gioendothelioma of soft tissue. Fine needle aspiration cytology, histology, electron microscopy and immunohistochemistry of a case. *Acta Cytol* 1986;30(2):194-200.
148. Minimo C, Zakowski M, Lin O: Cytologic findings of malignant vascular neoplasms: A study of twenty-four cases. *Diagn Cytopathol* 2002;26(6):349-355.
149. Boucher LD, Swanson PE, Stanley MW, et al.: Cytology of angiosarcoma. Findings in fourteen fine-needle aspiration biopsy specimens and one pleural fluid specimen. *Am J Clin Pathol* 2000;114(2):210-219.
150. Liu K, Layfield LJ: Cytomorphologic features of angiosarcoma on fine needle aspiration biopsy. *Acta Cytol* 1999;43(3):407-415.
151. Wakely PE Jr, Frable WJ, Kneisl JS: Aspiration cytopathology of epithelioid angiosarcoma. *Cancer* 2000;90(4):245-251.
152. Gherardi G, Rossi S, Perrone S, Scanni A: Angiosarcoma after breast-conserving therapy: Fine-needle aspiration biopsy, immunocytochemistry, and clinicopathologic correlates. *Cancer* 2005;105(3):145-151.
153. McCluggage WG, Sloan S, Kenny BD, et al.: Fine needle aspiration cytology (FNAC) of mammary granular cell tumour: a report of three cases. *Cytopathology* 1999;10(6):383-389.
154. Sirgi KE, Sneige N, Fanning TV, et al.: Fine-needle aspirates of granular cell lesions of the breast: Report of three cases, with emphasis on differential diagnosis and utility of immunostaining for CD68 (KP1). *Diagn Cytopathol* 1996;15(5):403-408.
155. Wiecek TJ, Krane JF, Domanski HA, et al.: Cytologic findings in granular cell tumors, with emphasis on the diagnosis of malignant granular cell tumor by fine-needle aspiration biopsy. *Cancer* 2001;93(6):398-408.
156. Liu K, Madden JF, Olatidoye BA, Dodd LG: Features of benign granular cell tumor on fine needle aspiration. *Acta Cytol* 1999;43(4):552-557.
157. Tos AP: Classification of pleomorphic sarcomas: Where are we now? *Histopathology* 2006;48(1):51-62.
158. Fletcher CD: Pleomorphic malignant fibrous histiocytoma: Fact or fiction? A critical reappraisal based on 159 tumors diagnosed as pleomorphic sarcoma. *Am J Surg Pathol* 1992;16(3):213-228.
159. Fletcher CD: The evolving classification of soft tissue tumours: An update based on the new WHO classification. *Histopathology* 2006;48(1):3-12.
160. Faquin WC, Pilch BZ, Keel SB, Cooper TL: Fine-needle aspiration of dedifferentiated chondrosarcoma of the larynx. *Diagn Cytopathol* 2000;22(5):288-292.
161. Layfield LJ, Liu K, Dodd LG, Olatidoye BA: "Dedifferentiated" chordoma: a case report of the cytomorphologic findings on fine-needle aspiration. *Diagn Cytopathol* 1998;19(5):378-381.
162. Dei Tos AP: Liposarcoma: New entities and evolving concepts. *Ann Diagn Pathol* 2000;4(4):252-266.
163. Binh MB, Sastre-Garau X, Guillou L, et al.: MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: A comparative analysis of 559 soft tissue neoplasms with genetic data. *Am J Surg Pathol* 2005;29(10):1340-1347.
164. Corradi D, Maestri R, Palmisano A, et al.: Idiopathic retroperitoneal fibrosis: Clinicopathologic features and differential diagnosis. *Kidney Int* 2007;72(6):742-753.
165. Dash RC, Liu K, Sheafor DH, Dodd LG: Fine-needle aspiration findings in idiopathic retroperitoneal fibrosis. *Diagn Cytopathol* 1999;21(1):22-26.
166. Stein AL, Bardawil RG, Silverman SG, Cibas ES: Fine needle aspiration biopsy of idiopathic retroperitoneal fibrosis. *Acta Cytol* 1997;41(2):461-466.
167. Dodd LG, Martinez S: Fine-needle aspiration cytology of pseudosarcomatous lesions of soft tissue. *Diagn Cytopathol* 2001;24(1):28-35.

168. Dahl I, Akerman M: Nodular fasciitis a correlative cytologic and histologic study of 13 cases. *Acta Cytol* 1981;25(3):215-223.
169. Plaza JA, Mayerson J, Wakely PE Jr: Nodular fasciitis of the hand: A potential diagnostic pitfall in fine-needle aspiration cytopathology. *Am J Clin Pathol* 2005;123(3):388-393.
170. Wong NL: Fine needle aspiration cytology of pseudo-sarcomatous reactive proliferative lesions of soft tissue. *Acta Cytol* 2002;46(6):1049-1055.
171. Pisharodi LR, Cary D, Bernacki EG Jr: Elastofibroma dorsi: diagnostic problems and pitfalls. *Diagn Cytopathol* 1994;10(3):242-244.
172. Domanski HA, Carlen B, Sloth M, Rydholm A: Elastofibroma dorsi has distinct cytomorphologic features, making diagnostic surgical biopsy unnecessary: Cytomorphologic study with clinical, radiologic, and electron microscopic correlations. *Diagn Cytopathol* 2003;29(6):327-333.
173. Bardin RL, Barnes CE, Stanton CA, Geisinger KR: Soft tissue amyloidoma of the extremities: A case report and review of the literature. *Arch Pathol Lab Med* 2004;128(11):1270-1273.
174. Dundore PA, Aisner SC, Templeton PA, et al.: Nodular pulmonary amyloidosis: Diagnosis by fine-needle aspiration cytology and a review of the literature. *Diagn Cytopathol* 1993;9(5):562-564.

Laboratory Management

EDMUND S. CIBAS

AGENCIES AND ORGANIZATIONS

Centers for Medicare and Medicaid Services
Joint Commission
College of American Pathologists
Council for Accreditation of Allied Health
Education Programs
Occupational Safety and Health
Administration
National Fire Protection Association

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of 1988
Health Insurance Portability and Accountability
Act of 1996

LABORATORY PERSONNEL

Laboratory Director
Technical Supervisor
General Supervisor
Cytotechnologist

POLICY AND PROCEDURE MANUALS

WORKFLOW

BILLING

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Codes
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The Screening (Routine) Pap Test
The Screening (High-Risk) Pap Test
The Diagnostic Pap Test
Coding Nongynecologic, Non-Fine-Needle
Aspiration Cases
Coding Fine-Needle Aspirates
Coding Consultation Cases

QUALITY CONTROL AND QUALITY ASSURANCE

Prospective 10% Rescreen
Retrospective Rescreen ("5-Year
Lookback")
Cytologic-Histologic Correlation
Statistics
Workload Records

PROFICIENCY TESTING

PERFORMANCE EVALUATION

Measures of Cytotechnologist
Performance
Screening Skills
Interpretive Skills
Measures of Cytopathologist
Performance
Atypical Squamous Cell-to-Squamous
Intraepithelial Lesion Ratio
High-Risk Human Papillomavirus-Positivity
Rates for Atypical Squamous Cells of
Undetermined Significance
Cytology-Biopsy Correlation

SAFETY

OSHA Bloodborne Pathogens Standard
Exposure Control
Hepatitis B Vaccination
Communication of Hazards to Employees
Recordkeeping
OSHA Laboratory Standard
National Fire Protection Association Standard
for Health Care Facilities
National Fire Protection Association Standard
on Fire Protection for Laboratories Using
Chemicals

In the United States, the cytology laboratory is one of the most regulated of all the laboratories involved in clinical testing. To effectively manage and work in a cytology laboratory, personnel must be familiar with the relevant regulatory agencies and professional organizations ("the players") and their licensure, accreditation, quality control, billing, and safety regulations ("the rules").

AGENCIES AND ORGANIZATIONS

Centers for Medicare and Medicaid Services

The United States does not have a national, single-payer health insurance system. Rather, U.S. citizens obtain health insurance from a variety of federal or private

carriers. The Centers for Medicare and Medicaid Services (CMS), formerly called the Health Care Financing Administration (HCFA), is a federal agency that provides health insurance to qualified individuals through the **Medicare** and **Medicaid** programs. Together, Medicare and Medicaid provide health care to 1 in 4 Americans. Besides Medicare and Medicaid, CMS also administers the **Clinical Laboratory Improvement Amendments (CLIA)** and **Health Insurance Portability and Accountability Act (HIPAA)** programs, and some other national health programs.

CMS is 1 of 11 divisions of the U.S. Department of Health and Human Services (DHHS). Some of the other divisions of DHHS are the National Institutes of Health, the Food and Drug Administration, and the Centers for Disease Control and Prevention (CDC). DHHS is a Cabinet-level department created in 1953, originally as the Department of Health, Education, and Welfare (HEW). In 1979, HEW became DHHS when the Department of Education split off as a separate department.

Joint Commission

The Joint Commission, formerly called the Joint Commission on Accreditation of Healthcare Organizations, is an independent, not-for-profit organization that evaluates and accredits more than 15,000 health care organizations in the United States. It is governed by a Board of Commissioners that includes physicians, consumers, and administrators. Its corporate members include professional societies like the American Medical Association (AMA) and the American Hospital Association.

Founded in 1951, the Joint Commission has developed standards for evaluating hospitals, assisted living facilities, outpatient services, and clinical laboratories. It has been accrediting hospitals for more than 50 years and laboratories since 1979. Laboratories surveyed by the Joint Commission have been deemed certifiable under Clinical Laboratory Improvement Amendments of 1988 (CLIA 88) requirements. To earn accreditation, a hospital or laboratory undergoes an on-site survey. To maintain accreditation, hospitals are surveyed every 3 years and laboratories every 2 years. Surveys have been unannounced since 2006.

In 2004, the Joint Commission initiated a new survey process that uses patient “tracers,” an evaluation method that focuses on service processes and traces patients through the care they have received. Surveyors also conduct systems tracers to analyze key operational systems that directly impact the quality and safety of patient care. The new process has shifted the emphasis from survey preparation to continuous improvement of operational systems that enhance safety and reduce medical errors.

College of American Pathologists

The College of American Pathologists (CAP) is a professional society of pathologists that offers a Laboratory Accreditation Program that inspects more than 6000 laboratories in the United States. Surveys are performed every 2 years and have been unannounced since 2006. Volunteer surveyors use inspection checklists (available online from the CAP Web site) that undergo regular revision to reflect federal regulations and professional standards. Like those inspected by the Joint Commission, CAP-inspected laboratories are eligible for CLIA certificates.

Council for Accreditation of Allied Health Education Programs

Council for Accreditation of Allied Health Education Programs (CAAHEP), formerly called the Committee on Allied Health Education and Accreditation (CAHEA), is an independent certifying agency for health education programs. It accredits cytotechnology training programs in the United States on the recommendation of the Cytotechnology Programs Review Committee of the American Society of Cytopathology.

Occupational Safety and Health Administration

Occupational Safety and Health Administration (OSHA) was created by Congress in 1971 under the Occupational Safety and Health Act, also known as “the safety bill of rights,” a response to public outcry in the 1960s against rising workplace-related injuries and deaths. A division within the Department of Labor, OSHA’s mission is to prevent injuries, illnesses, and deaths on the job. OSHA conducts inspections in response to reports of high injury rates or imminent dangers; fatalities or serious accidents; and employee complaints. Violations of standards can result in stiff monetary penalties.

Of particular relevance to cytology laboratories are the **OSHA Bloodborne Pathogens Standard** and the **OSHA Laboratory Standard**, which are available on the OSHA Web site.

National Fire Protection Association

Despite its name, the National Fire Protection Association (NFPA) is an international nonprofit organization founded in 1896. It represents close to 100 nations and serves as the premier advocate of fire protection. Its safety codes influence the design and construction of every building in the United States. A cytologist must be familiar with applicable safety codes to ensure that they are being observed, particularly when renovations to a laboratory are contemplated.

Of the numerous standards published by NFPA, the two of greatest relevance to cytology laboratories are the **Standard for Health Care Facilities** and the **Standard on Fire Protection for Laboratories Using Chemicals**. Both can be purchased from the NFPA Web site.

REGULATIONS

Clinical Laboratory Improvement Amendments of 1988

In the 1980s, there was an extraordinary flurry of media attention on the problem of false-negative Papanicolaou tests (Paps). The sentinel article appeared on the front page of the *Wall Street Journal*.¹ These media reports were a driving force behind legislation enacted by the U.S. Congress called the Clinical Laboratory Improvement Amendments of 1988 (CLIA 88). (A less stringent act, CLIA 67, had been passed in the 1960s, which mandated rescreening of 10% of negative Paps.) The final regulations of CLIA 88 were published on February 28, 1992, and minor modifications appeared on January 24, 2003.² They can be found at www.cdc.gov/clia/regs/toc.aspx. Congress charged CMS with the implementation of these standards.

Clinical Laboratory Improvement Amendments of 1988 highlights:

- workload limits for cytotechnologists
- new quality control (QC) procedures
 - 5-year retrospective rescreening
 - cytologic-histologic correlation
- pathologist review of:
 - all abnormal Paps, and “reactive and reparative changes”
 - all nongynecologic specimens
- proficiency testing
- unannounced specialized surveys

To bill for and receive Medicare or Medicaid payments, a clinical laboratory must have a CLIA certificate. To obtain a certificate, a laboratory must be accredited by one of two approved accrediting organizations, the Joint Commission or the CAP. In a few states like Washington and New York, a laboratory may obtain a state license in lieu of a CLIA certificate.³

Specialized surveys, required by CLIA 88, are performed by the American Society for Cytotechnology (ASCT) on a small proportion of cytology laboratories with CLIA certificates.⁴ A laboratory is selected either at random or if a complaint has been lodged against it with the CMS.³ The survey team evaluates the laboratory's operations and reviews at least 100 Pap cases. If applicable, a statement of deficiencies is forwarded to the

laboratory via the CMS regional office, and the laboratory is given an opportunity to respond with a plan of correction. Between 1988 and 2006 a total of 616 laboratories were surveyed. A significant proportion (32%) were found to be noncompliant with CLIA, but ultimately only 4% received sanctions, had their certificates revoked, or voluntarily withdrew from the program.⁵

An advisory committee known as the Clinical Laboratories Improvement Advisory Committee (CLIAC) consists of 20 members, including laboratorians and consumer advocates. CLIAC meets at least twice yearly and advises CMS and other governmental agencies on the need for and nature of any revisions to the standards that govern laboratory testing.

Health Insurance Portability and Accountability Act of 1996

HIPAA is a comprehensive law that regulates several unrelated areas of health care, like the protection of (1) health care coverage for those who change jobs and (2) the privacy of medical information. HIPAA asked Congress to pass comprehensive privacy legislation by August 1999. Because Congress did not do so, the law required DHHS to write the privacy rule/regulation. DHHS published the final rule on December 28, 2000, which took effect on April 14, 2001, requiring compliance by health care providers by April 14, 2003.

The rule governs the use of so-called “individually identifiable” health information and is intended to ensure that a patient's health information is used only for health purposes unless permission is obtained for other purposes. This information includes, for example, a cytologic diagnosis linked to an identifier such as a social security number, medical record number, or accession number. The DHHS Office of Civil Rights (OCR) is responsible for implementing the rule and has issued written guidelines for health care providers, which can be viewed at www.hhs.gov/ocr/hipaa.

HIPAA also requires that every provider who does business electronically use the same health care transactions, code sets, and identifiers. Code sets (e.g., CPT, HCPCS, and ICD-9) are the codes used to identify specific diagnoses and procedures on claims and encounter forms.

LABORATORY PERSONNEL

Personnel in a cytology laboratory include the cytotechnologists (CTs), cytopathologists (CPs), cytopreparatory staff, administrative staff, and clerical personnel. The qualifications and responsibilities of some of these have been defined by CLIA 88. Many of the job titles codified by CLIA, particularly “technical supervisor,” sound strange to cytologists's ears because they were not in

common usage before CLIA. In addition, the CLIA personnel designations often do not coincide with institution-based job titles, which, in many cases, predate CLIA 88 and were not changed to conform to CLIA titles, creating a confusing situation that often means an individual has one CLIA-defined title but a different institution-designated title.

Laboratory Director

Every laboratory performing so-called “high complexity” testing (cytology falls into this category under CLIA 88 regulations) must have a laboratory director. He or she may direct more than one laboratory, but no more than five. It is the laboratory director who has ultimate responsibility for the work performed in the laboratory.

Responsibilities of the laboratory director:

- overall operation and administration, including employment of personnel
- may also perform duties of the technical supervisor and general supervisor

Qualifications of the laboratory director:

- licensed to practice medicine or osteopathy
- anatomic pathologist or if not, must employ an anatomic pathologist as technical supervisor
- must possess a license as a laboratory director issued by the state in which the laboratory is located, if such licensing is required

The laboratory director of a cytology laboratory need not have specialty qualification (“boards”) in cytopathology.

Technical Supervisor

The technical supervisor (TS), as defined by CLIA 88, is the pathologist responsible for the “technical and scientific” oversight of the laboratory. He or she is not required to be on site at all times, but he or she must be available on an as-needed basis. The TS may perform the duties of a general supervisor and a CT.

Responsibilities of the technical supervisor:

- establishing QC procedures
- resolving technical problems
- evaluating competency of personnel
- monitoring test results
- performing semiannual evaluations of CTs
- establishing a workload limit for each CT and reassessing it at least every 6 months

The TS may delegate some of his or her responsibilities to a trainee in the final year of training in anatomic pathology.

Qualifications of the technical supervisor:

- licensed to practice medicine or osteopathy
- certified in anatomic pathology, or
- certified by the American Society of Cytopathology to practice cytopathology

The American Society of Cytopathology no longer certifies CPs; the last examination was in 1978. Like the laboratory director, the TS need not have specialty qualification (boards) in cytopathology. In any given laboratory, more than one individual may qualify as a TS, but one is usually designated as the TS. In many cytology laboratories, the role of the TS is performed by the laboratory director.

General Supervisor

Each cytology laboratory must have a general supervisor (GS). The GS is responsible for the day-to-day oversight of operations and personnel. He or she must be accessible to provide on-site, telephone, or electronic consultation to resolve problems in accordance with procedures established by the TS. The same individual may fulfill the roles of both TS and GS.

Qualifications of the general supervisor:

- same as TS, or
- CT (see qualifications herein) with at least 3 years of full-time experience within the past 10 years

Cytotechnologist

The job description of a CT varies depending on the laboratory where the CT works. Most CTs are involved in slide examination. In some laboratories, CTs are involved in QC activities, teaching, research, cytopreparation, and assistance with preparation of smears and adequacy assessments during fine-needle aspirations (FNAs). The following responsibilities of a CT are the minimum established by CLIA 88.

Responsibilities of the cytotechnologist (defined by Clinical Laboratory Improvement Amendments):

- documenting slide interpretation results
- recording the number of slides examined each day
- recording the number of hours worked each day

Qualifications of the cytotechnologist (at least one of the following):

- graduated from a school of cytotechnology accredited by CAAHEP
- certified in cytotechnology by an approved agency (American Society of Clinical Pathologists or HEW)
- “grandfathered” based on experience. (Opportunities for “grandfathering” ended on September 1, 1994.)

CTs are not required under CLIA 88 to be certified by a certifying agency, so long as they have graduated from an approved school. The Board of Registry (BOR) of the American Society of Clinical Pathologists, which certifies CTs, offers two certificates: CT and specialist in cytotechnology (SCT). The SCT certificate is given to those who pass a higher level examination. The BOR used to offer an “experience route,” but this is no longer the case, and CTs who have not attended an approved school of cytotechnology can no longer sit for the BOR examination. As of 1988, a bachelor’s degree, plus graduation from an accredited program, is required for eligibility for a certificate.

HEW once offered certification, but this ended many years ago. Certification by the International Academy of Cytology (IAC) of foreign-trained CTs is not sufficient for those who wish to practice in the United States because the IAC is not an approved certifying agency.

POLICY AND PROCEDURE MANUALS

The cytology laboratory is responsible for maintaining two types of procedure manuals, a client service manual and a laboratory procedure manual. The client service manual is a written or electronic guide to providers on proper methods for obtaining, storing, and transporting specimens to the cytology laboratory.

Client service manual—required policies:

- preparation of patients
- specimen collection
- specimen labeling
- specimen preservation
- conditions for transportation

For example, policies specify when refrigeration is recommended for body cavity fluids and how to obtain a proper urine sample for cytology.

Every laboratory must also maintain a laboratory procedure manual. The Clinical and Laboratory Standards Institute (CLSI), formerly the National

Committee for Clinical Laboratory Standards (NCCLS), publishes a document that outlines steps for preparing and maintaining such manuals.⁶ It does not, however, address many of the specific policies and procedures of cytology laboratories. Tips for compiling a cytology laboratory procedure manual can be found in other references.^{7,8}

Laboratory procedure manual:

The procedure manual must include:

- requirements for specimen collection and processing
- criteria for specimen rejection
- procedures for microscopic examination
- step-by-step description of the performance of a procedure

Procedures must be:

- approved, signed, and dated by the director
- reapproved, signed, and dated if the directorship changes

Each change in a procedure must be approved, signed, and dated by the director. Records of discontinued procedures must be kept for 2 years.

Textbooks and manufacturer’s procedure manuals can be used as references or supplements, but not as a replacement for written procedures. Electronic manuals are acceptable, so long as they are readily available.

WORKFLOW

The flow of work in a cytology laboratory follows an established pathway, and the CLIA 88 regulations specify certain requirements in the process for quality control purposes.

Steps in the flow of work of a cytology laboratory:

- specimen collection and transportation
- accessioning
- slide preparation
- slide examination
- reporting results
- record retention

To ensure proper handling of specimens and documentation, the CLIA 88 regulations specify certain mandatory procedures.

For starters, samples must be accompanied by a cytology requisition form that is completed by a physician or other authorized individual. *Requisitions* must be retained for at least 2 years.

The requisition must include:

- patient's name or other unique identifier
- patient's age or date of birth
- patient's gender
- name and address of the authorized person ordering the test, or the name and address of the laboratory submitting the specimen, with contact person
- date of specimen collection
- test to be performed
- specimen source
- for Pap tests:
 - last menstrual period
 - indication whether there has been a previous abnormal Pap, treatment, or biopsy
- any additional information relevant and necessary to a specific test to assure accuracy

The laboratory must have a policy that documents criteria for the rejection of specimens (e.g., broken slides, missing patient identifiers, or lack of medical necessity).

All Pap tests must be stained using a Papanicolaou or modified Papanicolaou staining method.

Measures must be in place to prevent cross-contamination between Paps and nongynecologic specimens during the staining process. In addition, nongynecologic specimens with a high potential for cross-contamination must be stained separately, and the stains filtered or changed after use. Direct smears from sediments of highly cellular specimens are especially problematic; cytocentrifuge, filter, and thinlayer preparations are less likely to lead to cross-contamination during staining. Highly cellular specimens can be identified using a toluidine blue or other rapid stain on a wet preparation.

All cytology slides must be evaluated on the premises of a laboratory certified for cytologic testing. Slides may not be taken home for evaluation. The test record system must include the identity of the personnel who performed the test, and the date and time the specimen was received.

CLIA 88 requires that a TS review every Pap interpreted as showing reactive or reparative changes, and any squamous or glandular abnormality at the level of atypical squamous cells (ASC) or atypical glandular cells (AGC) and higher. A TS does not need to review a case that a CT judges to be negative and lacking in reactive and reparative changes. In addition, a TS must review all nongynecologic cytology cases. A TS's written or electronic signature must be on the cytology report, and CLIA 88 specifies that the report must use narrative, descriptive terminology. A numerical reporting system (e.g., "Class IV") is not acceptable.

The test report must include:

- the name and address of the laboratory
- the test performed
- result
- pathologist signature

In case a corrected (amended) report needs to be issued, the corrected report must state the reason for the correction.

Reports must be retained for 10 years. This may be in electronic or hard copy format. *Cytology slides* must be retained for at least 5 years. The requirement is the same whether the sample is gynecologic or non-gynecologic, normal or abnormal. Table 17.1 summarizes the retention requirements for cytology records and slides. Note that these are federal regulations; state regulations may be more stringent. In Massachusetts, for example, cytology slides must be retained for 7 years.

TABLE 17.1 FEDERAL RETENTION REQUIREMENT FOR CYTOLOGY LABORATORIES

Requisitions	2 years
Worksheets*	2 years
Slides	5 years
Reports	10 years

* Includes quality control, quality assurance, and proficiency testing results.

BILLING

Billing is one of the most complex aspects of laboratory management in the United States. A cynic might say that the labyrinthine regulations were designed to be difficult so that payers could deny reimbursement to providers. Certainly, some of the rules defy logic, but, notwithstanding this, all providers or laboratories are bound to comply or else risk forfeiting reimbursement or incurring stiff penalties for fraud and abuse.

In this section, the rules for filing claims to government agencies for cytology-related services provided to Medicare, Medicaid, and TriCare beneficiaries are outlined.⁹ The claim policies of private insurers and managed care companies are likely to be different in some respects. Importantly, the rules are different for Pap tests, nongynecologic or non-FNA specimens, FNAs, and consultations, and therefore each will be discussed separately. But first the reader needs to understand the "languages" known as CPT and ICD-9, two different but complementary coding systems that are required for all medical billing in the United States. To bill for a test, the laboratory must submit a claim that includes (1) the date and location of service; (2) a procedure code (to describe what was done); and (3) an ICD-9 code (to justify medical necessity).

Procedure Codes

A medical bill submitted to an insurer for payment needs to describe the medical procedure or service that is being billed. The common language that is used in the United States to communicate the vast majority of procedures is called **current procedural terminology** or **CPT**, a registered trademark of the AMA. The AMA owns and maintains CPT. The first edition was published by the AMA in 1966 when the (then new) Medicare program needed a terminology for describing medical services. To this day, CMS, which administers the Medicare program for DHHS, agrees via contract with the AMA to use the CPT book as the main source of codes and descriptors for processing medical claims. With the implementation of the HIPAA regulations in 2003, CPT became the language that must be used by all providers, government agencies, and private insurers.

According to the AMA, the objective of CPT is to provide “a uniform language that will accurately describe medical, surgical, and diagnostic services, and will thereby provide an effective means for reliable nationwide communication among physicians, patients, and third parties.”¹⁰ A CPT code has been assigned to virtually every type of physician and laboratory service, including cytologic slide preparation and interpretation. (For example, CPT code 10021 describes the procedure of performing an FNA without image guidance.) CPT codes describe even the most complex of medical procedures in the form of a simple five-digit code. Tell a knowledgeable person, for example, that you just performed an 88164, and he or she will know immediately that this was a manual screening of a cervical or vaginal smear (not a liquid-based preparation); that Bethesda terminology was used to report the result; and that the procedure included only the so-called “technical” component (staining, coverslipping, CT review, but not a CP’s interpretation). All this from a five-digit code!

CPT codes are the foundation for determining facility (“technical”) and physician (“professional”) payments, in conjunction with Medicare’s Resource-Based Relative Value System (RBRVS). The RBRVS is a system for comparing the relative value of medical services across all specialties, based on work, practice expense, and other factors. By doing so, the RBRVS establishes a relative value unit (RVU) for every current medical procedure. The dollar value of any given medical service or procedure is determined by its composite relative weight, multiplied by a nationally set (by CMS) dollar conversion factor. The RVUs and conversion factor for physician services are published annually in the Federal Register by CMS. The conversion factor for 2007 was \$37.8975. Various geographic cost-of-living adjustments and other factors are also applied to obtain the specific allowed charge for any given procedure and locale, and therefore the process is not as simple as multiplying an RVU by the

conversion factor. (It is not within the scope of this chapter to elaborate on the detailed formula.) CMS provides an allowed charge lookup system on its Web site at www.cms.hhs.gov.

Healthcare Common Procedure Coding System (HCPCS) codes are a separate set of codes used to describe drugs, supplies, and certain other services not included in CPT. Like CPT codes, HCPCS codes have five digits, but the first is a letter and the rest are numbers (e.g., G0123). The HCPCS codes are administered not by the AMA but by the CMS. Responsibility for maintaining and updating them is vested in a national panel composed of representatives from CMS, the BlueCross BlueShield Association, and America’s Health Insurance Plans. Cytologists need to be concerned with only a small number of HCPCS codes, those for routine and high-risk Pap tests for Medicare beneficiaries.

In some circumstances, CPT and HCPCS codes require the use of modifiers to avoid filing a false claim and to assure prompt payment by payers. A complete discussion of modifiers is beyond the scope of this chapter, but familiarity with the concept of modifiers is important. Some commonly used modifiers for cytology cases deserve mention.

CPT Modifier 26. This is the most widely used in pathology. It denotes that only the physician professional component of the service is being billed.

CPT Modifier 52. This modifier denotes a reduced service from the customary procedure. In cytology, a good example is the review of a slide that was evaluated by the ThinPrep Imaging System but rejected for technical reasons. A laboratory can still bill the automated screening code 88175, but with modifier 52 (i.e., 8817552).

CPT Modifier 59. Modifier 59 denotes a “separate procedure,” such as a different specimen (e.g., washing versus brushing) or anatomic site. Payers often require this modifier when two or more codes are considered mutually exclusive or duplicative. For example, reporting 8810459 for a direct smear bronchial brushing with 88108 for a cytospin bronchial washing is often necessary to avoid having the former charge denied.

HCPCS Modifier GC. Teaching physicians must append modifier GC to CPT and HCPCS codes on Medicare claims when a resident or fellow actively participates in performing the underlying medical service. The modifier declares that the teaching physician personally performed the “critical” portion of the procedure and is thus entitled to bill for it.

HCPCS Modifiers GA, GY, and GZ. These modifiers are applied to Pap test HCPCS codes when billing Medicare. They clarify the laboratory’s right (or lack thereof) to bill the Medicare beneficiary for the charge if it’s denied by the contractor.

HCPCS Modifier TC. This modifier denotes the facility technical component of the service being billed, and thus is the counterpart of the CPT 26 modifier.

A few points about procedure codes are worth noting:

1. **Just because a code is printed in CPT or HCPCS does not mean it is a covered service.** Coverage decisions are made by the U.S. Congress, state legislatures, and private insurers. Coverage limits might also be imposed by participation agreements you make with managed care companies and private insurers.
2. **The AMA and CMS sometimes have conflicting interpretations on the scope and meaning of the CPT codes.** Historically, the AMA was the sole authority everyone, including Medicare, looked to for guidance in using CPT codes. In 1996, Medicare launched its National Correct Coding Initiative (NCCI). Since then, the AMA and CMS have diverged in ways that affect a number of pathology-related procedure codes. The result: “AMA-CPT rules” and “Medicare-CPT rules.” A good example are the nongynecologic cytology procedure codes 88104 (direct smears) and 88108 (cytospin). Medicare says it is not medically necessary to use both types of preparations for one nongynecologic cytology specimen, and therefore you are only permitted to bill 88108 to Medicare, even if you examined both preparations. To the contrary, the AMA considers both procedures billable, even when they relate to the same specimen. How should one deal with such discrepancies? You should adhere to CMS policy if billing a service to a Medicare carrier or fiscal intermediary (“render unto Caesar ...”). You should also adhere to CMS policy for Medicaid, TriCare, Medicare Advantage, and private insurer accounts if they specify that you should adhere to Medicare CPT policies. If they do not, follow their specific instructions (if any), or follow the AMA rules if the insurer does not name a CPT authority.
3. **You should always use only the most recent version of the CPT codebook.** The so-called “Category I” CPT codes that account for 99% of the codes you will use are updated effective January 1 every year, and every year some edits are made that affect pathology codes.

International Classification of Diseases (ICD)-9 Codes

The International Classification of Diseases, now in its ninth revision (ICD-9), is the taxonomy used by all health care professionals and insurers in the United States when discussing medical conditions.¹¹ (For example, in ICD-9

“speak,” hematuria is 599.7, and a solitary thyroid nodule is 241.0.) ICD-9 coding is used to determine whether or not a procedure billed to an insurer is medically necessary, in which case it is a covered benefit for the patient. With the passage of the Medicare Catastrophic Coverage Act of 1988, diagnostic coding using ICD-9 became mandatory for Medicare claims, and when HIPAA was implemented in 2003, ICD-9 coding became universal, meaning that private insurers and government agencies are required to use it.

In the United States, all health care providers must furnish an ICD-9 code to justify the medical necessity of a diagnostic test. For example, if a urine cytology test is ordered for a patient with hematuria, the patient’s physician will furnish ICD-9 code 599.7 (which denotes hematuria) on the requisition form that accompanies the urine specimen. Similarly, when your laboratory bills an insurer for having performed a cytologic examination of a specimen, the bill must include an appropriate ICD-9 code to justify the medical necessity of the test. (It might be the same “clinical code” you received from the ordering physician, or it might be different, a “pathologic code,” as discussed herein.) Payers have lists of approved ICD-9 codes for many laboratory tests and will deny payment if a nonapproved code is provided. In the preceding example, if you supply a wrong ICD-9 code, like 784.0 (denoting “headache”) with the bill for the urine cytology, it is likely that Medicare will deny the claim, because 784.0 does not justify the medical necessity of urine cytology. Pointers for selecting the appropriate ICD-9 code for cytology specimens are provided herein.

The provider who sends a cytology sample to the laboratory does not have to provide a literal ICD-9 code. It is acceptable for the referring physician to write a narrative diagnosis (e.g., “hematuria”) on the requisition form, which the laboratory can then translate into an ICD-9 code (in this example, 599.7) by consulting the codebook.

The ICD-9 codebook consists of three volumes. The entire three-volume set is used by hospitals to bill for *inpatient services*. All other providers, and hospitals billing for *outpatient services*, use only the first two volumes of the set. Volume one is a tabular list of hundreds of three-, four- and five-digit numeric codes, each accompanied by a description of the corresponding condition. Volume one is arranged by systems. Volume two is an alphabetical list of the conditions included in volume one. Volumes one and two are distributed by the National Center for Health Statistics, a branch of the CDC. You need to be alert for changes in the ICD-9 codes (i.e., watch the CMS and professional associations’ Web sites) because the codes are eligible for update twice a year (April 1 and October 1). Volume three, with rules for inpatient coding, are not of concern to pathologists and independent labs.

A few points about ICD-9 coding for nongynecologic cytology services, including FNAs, are worth noting:

1. **The pathologic diagnosis is the principal diagnosis used for ICD-9 coding.** For example, if you interpret a urine specimen as “positive for” or “consistent with” transitional cell carcinoma, the code for “transitional cell carcinoma, site not specified” (188.9) should be reported on the insurance claim. You can think of this as the “pathologic code,” as opposed to the “clinical code” that came from the patient’s attending physician.
2. **The clinical code is used for coding if there is no specific pathologic diagnosis.** In many circumstances, a specific pathologic diagnosis cannot be assigned to a specimen. This applies, for example, to a negative urine specimen. In this circumstance you must fall back on the clinical code that was provided with the specimen (e.g., 599.7 for hematuria).
3. **Do not report an uncertain diagnosis.** In cytology (or surgical pathology, for that matter), your interpretation will occasionally be “suspicious for” or “can’t rule out.” In such circumstances, do not report the uncertain diagnosis as if it were confirmed. For example, do not use code 193 (malignant neoplasm of the thyroid) if you interpret a thyroid FNA as “suspicious for papillary carcinoma.” Instead, code it to the highest degree of certainty for that patient visit (e.g., 241.0: solitary thyroid nodule).
4. **Report codes to the greatest level of specificity.** For example, there are ICD-9 codes for a malignant neoplasm of the upper (162.3), middle (162.4), and lower lobes (162.5) of the lung, and a code for an “unspecified” site (162.9). If you diagnose a small cell carcinoma in an FNA of an upper lobe lesion, use 162.3, not 162.9.

5. **As with the CPT codebook, always use the most recent version of the ICD-9 codebook.**

The ICD-9 codebook includes a series of so-called “health status” codes called “V codes” because they always start with the letter V. They are used when a patient receives health services in the absence of any current sign or symptom of disease or injury. One example is a Pap test for a healthy woman. In such a circumstance, you most likely will report a code like V72.6 because the Pap is normal, and the referring physician furnished no information regarding abnormal signs, symptoms, or history.

Coding Pap Tests

Because of recent advances in technology, it is impossible to talk about a generic Pap test, certainly with regard to procedure coding. One code (or even a few codes) would be inadequate to describe the variety of ways that a Pap might be evaluated today: as a smear or liquid-based preparation; manually or with computer assistance; computerized screening only or with manual review; with or without physician interpretation. Thus, assigning a procedure code to a Pap test follows a rather complex algorithm. As of this writing (2008), there are 14 different CPT codes for a Pap test, and a complementary set of HCPCS codes. A complete discussion of all the codes is beyond the scope of this chapter, but those most commonly used are described in [Table 17.2](#).

Anal Pap tests are considered nongynecologic specimens and are discussed as such.

Another aspect of Paps that makes them unique is that, most of the time, they are ordered as a screening test in the absence of any history, signs, or symptoms related to the cervix or vagina. For Medicare beneficiaries,

TABLE 17.2 PROCEDURE CODES FOR PAP TESTS (AS OF 2008)

Procedure Codes (HCPCS) for Screening Pap Tests (Routine and High-Risk)					
Preparation Method	Component	Manual Screening Only	ThinPrep Imager-Assisted Screening	FocalPoint (Instrument Only)	FocalPoint (with Manual Screening)
Smear	Technical	P3000	—	G0147	G0148
	Professional	P3001	—	G0141	G0141
Liquid-based	Technical	G0123	G0145	G0144	G0145
	Professional	G0124	G0141	G0124	G0141
Procedure Codes (CPT) for Diagnostic Pap Tests					
Preparation Method	Component	Manual Screening Only	ThinPrep Imager-Assisted Screening	FocalPoint (Instrument Only)	FocalPoint (with Manual Screening)
Smear	Technical	88164*	—	88147	88148
	Professional	88141	—	88141	88141
Liquid-based	Technical	88142	88175	88174	88175
	Professional	88141	88141	88141	88141

*Substitute 88150 if Bethesda System terminology is not used to report results.

CPT, current procedural terminology; HCPCS, healthcare common procedure coding system.

Pap tests are therefore divided into three categories according to medical indication, thus significantly affecting CPT/HCPCS procedure and ICD-9 diagnosis coding. These categories have implications for the frequency of beneficiary coverage and direct payment to the laboratory by Medicare (as opposed to beneficiary financial liability), and thus the categories affect procedure coding, as seen in [Table 17.2](#).

The three categories of Pap tests and their implications for Medicare beneficiary coverage are:

- screening, routine: no more than one test every 24 months is covered
- screening, high risk: no more than one test every 11 months is covered
- diagnostic: unlimited coverage

The coding rules for these three categories of Pap tests are described in the sections that follow. Note that most Medicaid agencies and private insurers do not differentiate Pap tests to the same degree or in the same way as does Medicare.

The Screening (Routine) Pap Test

The “routine screening Pap test” is defined by Medicare as follows.

Definition of a screening (routine) Pap test:

- no current sign, symptom, or complaint referable to the cervix
- no previous abnormal Pap
- no high-risk factors for cervical cancer

A screening (routine) Pap is one ordered solely as part of a preventive health care visit (e.g., annual or other periodic check-up). If the woman has not had a Pap paid for by Medicare within the past 2 years, the laboratory can expect payment by Medicare and should post one of the four accepted ICD-9 codes **V72.31**, **V76.2**, **V76.47**, or **V76.49** (see [Table 17.3](#) for definitions) as it has been supplied (hopefully!) by the referring physician. (Should the pathologist report an abnormality not expected by the referring physician, the ICD-9 code for that atypia would be posted as a secondary diagnosis on the claim. This is how Medicare becomes aware that the beneficiary is to move to a high-risk or diagnostic category because in the future she will have a history of an abnormal Pap.) If the woman has already had a Pap paid for by Medicare within the past 2 years, the test is not payable by Medicare. The laboratory may then bill the patient directly, but only if it has a signed Advance Beneficiary Notice (ABN) from the patient on file. By signing an ABN, the patient authorizes the laboratory to bill her directly if a service is not covered by Medicare.

TABLE 17.3 COMMONLY USED ICD-9 CODES FOR PAP TESTS

Code	Definition
V76.2	Screening Pap of cervix in the absence of signs, symptoms, or history
V72.31	Screening Pap of cervix in the absence of signs, symptoms, or history, collected as part of gynecologic examination
V76.47	Screening Pap of vagina in the absence of signs, symptoms, or history
V76.49	Screening Pap in the absence of signs, symptoms, or history, woman without a cervix
V15.89	Screening Pap, woman at high risk for developing cervical or vaginal cancer
616.0	Cervicitis
616.10	Vaginitis
622.10	Histologic CIN, unspecified
622.11	Histologic CIN I (mild dysplasia)
622.12	Histologic CIN II (moderate dysplasia)
233.1	Histologic CIN III (severe dysplasia)
795.00	Abnormal glandular Pap (cervix)
795.01	Abnormal cervical Pap—ASCUS
795.02	Abnormal cervical Pap—ASC-H
795.03	Abnormal cervical Pap—LSIL
795.04	Abnormal cervical Pap—HSIL
795.05	High risk HPV DNA positive (cervix)
795.06	Malignant cells (cervical Pap) without histologic confirmation
795.08	Unsatisfactory cervical Pap
795.09	Other abnormal cervical Pap (includes reactive or reparative changes)
112.1	Candidiasis
623.5	Vaginal discharge
626.2	Menometrorrhagia
626.4	Irregular menstrual cycle
626.6	Metrorrhagia
626.8	Dysfunctional uterine bleeding
627.1	Postmenopausal bleeding

ASC-H, atypical squamous cells, cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

The laboratory generally relies on the referring physician to obtain the ABN signatures it needs because it almost never has direct contact with the patient in advance of a Pap test. (Medicare states that an ABN is valid—and the patient is financially liable for the service—only if the ABN is signed in advance of the test; post-test ABN signatures are not valid or binding on the beneficiary.) It is advisable, however, not to rely on the referring physician to store ABNs for you. Many laboratories have solved this problem by making the ABN a part of the requisition form itself.

Laboratories frequently append modifier GA or GZ to the HCPCS code when billing Medicare for a screening or high-risk Pap test. The modifiers declare whether or not the laboratory has an ABN on file to permit billing the beneficiary for the charge if Medicare denies it. Modifier GA says that a beneficiary-signed ABN is available, so the laboratory can bill the beneficiary if Medicare denies the charge. Modifier GZ says the laboratory did not obtain an ABN, so the beneficiary cannot be billed if Medicare denies the charge. Consult with your compliance officer for additional information about modifiers GA and GZ, and modifier GY, which is not herein explained because of infrequent usage by laboratories.

The laboratory is responsible for ensuring that physicians distribute an ABN advisory to all Medicare patients informing them that Medicare does not pay for all laboratory tests ordered under all circumstances.

Medicare expects one of the four V-codes to appear on the laboratory's claim for a routine screening Pap. The referring physician is not always so cooperative as to provide one of those codes on the requisition form. In these instances, the laboratory is advised to contact the referring physician to get a conforming ICD-9 code because the laboratory could be charged with filing a false claim if it unilaterally adds a missing code or changes a code provided by the referring physician. Similarly, if the routine screening Pap shows an unexpected abnormality, the referring physician can be contacted to determine if something in the patient's history or current visit might have been overlooked when ordering the test; otherwise, Medicare may not cover the test, even though it turned out to be abnormal. ICD-9 reporting tips for routine screening Pap tests are provided below.

The Screening (High-Risk) Pap Test

The screening (high-risk) Pap test is defined by Medicare as follows.

Definition of a screening (high-risk) Pap test:

- early onset of sexual activity (under 16 years of age)
- multiple sexual partners ($\geq$ five in a lifetime)
- history of sexually transmitted disease (including human immunodeficiency virus [HIV])
- fewer than three negative Paps in the previous 7 years
- daughter of a woman given diethylstilbestrol during pregnancy
- abnormal Pap in the past 3 years (childbearing age women only)

As you can see from the definition, to properly assign a clinical ICD-9 code when submitting a Pap to a laboratory, the referring physician must obtain a detailed history of risk factors. The clinical code for a high-risk

Pap is V15.89, with one exception: if the high-risk factor is early onset of sexual activity, the ICD-9 code is V69.2. These codes must be supplied by the referring physician, not assigned unilaterally by the laboratory. Medicare will cover the test (i.e., pay the laboratory directly) only if it has not paid for another Pap for the patient within the past 11 months. If it has, the test is not covered, and the laboratory can bill the patient directly, provided it has a properly executed ABN form on file.

The Diagnostic Pap Test

The diagnostic Pap test is defined by Medicare as follows.

Definition of a diagnostic Pap test:

- previously diagnosed cancer of the vagina, cervix, or uterus
- previous abnormal Pap
- current abnormal findings of vagina, cervix, uterus, ovaries, or adnexa
- significant complaint referable to the female reproductive system
- any sign or symptom that might be related to a gynecologic disorder

As defined, a diagnostic Pap test is always covered by Medicare, whether or not it is abnormal, so long as the clinical ICD-9 code provided by the referring physician is on the local Medicare Part B carrier's limited coverage list. Medicare carriers publish their local medical review policies (called local coverage determinations [LCDs]) in bulletins and their Web sites. An LCD contains a list of ICD-9 codes that justify the medical necessity of a Pap test in the opinion of the local Medicare Part B carrier. (Ironically, even though Medicare is a national program, it happens all too often that a Pap or other laboratory test that is covered in one carrier's jurisdiction is not covered in another locale!) Some commonly accepted diagnostic Pap test ICD-9 codes are listed in [Table 17.3](#).

A few points worth noting regarding Pap test coding:

1. **Only the referring physician can classify a Pap as screening, high risk, or diagnostic.** The laboratory has no right to unilaterally classify the Pap or reassign it to a different class than that given by the referring physician. This noncompliant activity is equivalent to "code jamming." Nevertheless, the laboratory may have information (previous medical history) available to it that suggests that the referring physician has misclassified the medical necessity of the test. Under these circumstances, the laboratory may amend the order but only after contacting the referring physician and obtaining authorization to do so.

2. **ICD-9 coding rules for screening (routine and high-risk) Paps include instructions for “primary” and “secondary” codes.** When submitting a claim to Medicare for a screening Pap test (either routine or high risk), the clinical ICD-9 code submitted by the ordering physician (typically a V-code as previously enumerated) must appear in claim field position one (i.e., the “primary” diagnosis”). If an abnormality is discovered after cytologic review of the slide, the ICD-9 code for that abnormality is reported in claim field position two (i.e., a “secondary” diagnosis). As mentioned, this is how Medicare becomes aware that the beneficiary is to move to the high-risk or diagnostic category because in the future she will have a history of an abnormal Pap.

Official ICD-9 guidebook reporting instructions for screening tests are fundamentally the same as were just described for Medicare. Therefore, for a non-Medicare patient, you should report the screening code (V-code) supplied by the ordering physician as the primary diagnosis and report any abnormality determined during the laboratory examination process as a secondary diagnosis. It is conceivable that a Medicaid agency or private insurer may, however, prescribe a different reporting protocol.

3. **You can bill for an unsatisfactory Pap under certain circumstances.** A small percentage of Paps are reported as “Unsatisfactory,” usually by a CT. If the slide was deemed unsatisfactory before it was reviewed (broken beyond repair, insufficient identifying information, etc.), a charge should not be submitted on that case. If, however, the slide was reviewed and deemed unsatisfactory because of obscuring blood, insufficient squamous cells, etc., then a charge should be reported, accompanied by ICD-9 code 795.08 (if cervical Pap) or 795.18 (if vaginal Pap).
4. **A physician interpretation charge should only be billed if the pathologist interpreted the slide as a result of an atypia or question identified by the CT.** When a pathologist interprets or reviews a Pap, his or her signature (handwritten or electronic) must appear on the report. Conversely, the pathologist's signature should not appear on a Pap report when he or she did not personally review the slide. (The pathologist's name, as lab director or staff, may appear in the masthead of the report alongside the name and address of the laboratory, so long as it is clear that it is not a signature.) A separate professional fee is billable by the pathologist if his or her interpretation is precipitated by a finding of abnormal, atypical, or reactive cells by the CT. A pathologist's professional fee is legitimate in this instance even though he or she ultimately signs out the smear as “normal.” Pathologist review of a high-risk patient smear judged to be normal by the

CT is not a separately billable professional service; similarly, a pathologist review that is conducted as part of the laboratory's quality assurance and quality control program is not separately billable. When a pathologist screens a smear in place of a CT and no abnormality or atypia is identified, the test is billable with the regular technical code (e.g., 88142, G0142) alone.

Coding Nongynecologic, Non-Fine-Needle Aspiration Cases

This section focuses on all nongynecologic cytology specimens, excluding FNAs. (The rules for FNA billing are different and discussed in the next section.) Anal Pap tests are considered nongynecologic specimens and therefore coded according to the rules in this section.

The rules for coding nongynecologic or non-FNA specimens are much simpler than those for Paps. The procedure (charge) codes are based not on specimen type (e.g., urine, cerebrospinal fluid [CSF], bronchial brushing), but on the preparation method, and the choices are rather limited (Table 17.4). Each separate nongynecologic or non-FNA specimen is assigned one or more procedure codes, depending on the preparations that are ordered by the pathologist. Direct smears, cytopins, thinlayer smears, and cell block slides are distinct preparations and are separately coded per specimen. Thus, a nongynecologic and non-FNA specimen prepared as one or more direct smears is coded as **88104**. The cytopin and Saccomanno methods are considered “concentration” methods (code **88108**), and the ThinPrep® and SurePath® nongynecologic preps are considered “enriched/concentration” methods (code **88112**). A cell block has a separate charge code (**88305**).

Unlike some surgical pathology specimens, in which certain separately identified specimens cannot be billed

TABLE 17.4 PROCEDURE CODES FOR NON-GYNECOLOGIC, NON-FINE-NEEDLE ASPIRATION SPECIMENS

Preparation	Code
Direct smear	88104
Concentrated prep (cytopin or Saccomanno)	88108
Enriched or concentrated prep (e.g., ThinPrep®, SurePath®)	88112
Cell block	88305
“Other source” (received as stained smear)	88160
“Other source” (received as unstained smear)	88161
Extended cytology study of “other source” specimen	88162
Special stains for microorganisms	88312
Other histochemical stains (e.g., periodic acid-Schiff)	88313
Immunohistochemical stains	88342

separately but must be “bundled” (e.g., breast and axillary lymph nodes from a modified radical mastectomy procedure), rarely are cytology samples bundled. For example, right and left pleural fluids from the same patient received on the same day are billed separately, as are bronchial brushings and washings. Bundling is required only in those unusual situations when a sample like sputum (or bronchial washings from the same site) arrives in multiple vials; there is no clinical basis for splitting such a sample, and charge reporting is impacted accordingly. But if two samples are submitted from the same patient on the same day, and they are *from different sites*, they can and should be billed as separate specimens.

In many cases, multiple preparations are made, and a charge code for each is appropriate, except when the patient is a Medicare beneficiary. For example, if a pleural fluid is prepared as a smear, cytopsin, and cell block, codes 88104, 88108, and 88305 are considered appropriate by the AMA. But Medicare permits billing only the 88108 and 88305 codes in this instance; the 88104 code is considered medically unnecessary in light of the 88108. (This is an example of the dichotomy between AMA-CPT rules and Medicare-CPT rules.) The AMA and CMS agree, however, that you cannot report 88112 and 88108 together for the same specimen because 88112 already includes concentration.

A small number of cytology specimens are considered to be “other source” nongynecologic samples in CPT parlance. These are, principally, sputum, nipple discharge, and anal-rectal cytology. If one of these specimens arrives as a stained slide or slides (presumably a rare event), report **88160**; but if the slide(s) is stained in your lab, report **88161** for the specimen. Note that 88160 or 88161 replaces 88104 for an “other source” specimen only if the preparation is a direct smear; report the regular 88108 or 88112 code if a concentrated or enriched or concentrated preparation is made. There’s also an “extended study” “other source” code, **88162**, but the circumstances under which it may be reported seldom occur these days.

A few additional points are worth noting regarding coding nongynecologic or non-FNA specimens:

1. **The number of smears, cytopsins, ThinPreps, or SurePath slides you examine for a specimen does not affect the charge code.** Codes 88104, 88108, and 88112 are applied once per specimen no matter how many slides of each preparation type were examined. These codes can be used in combination (with certain restrictions, as mentioned previously), but they cannot be multiplied for any one specimen.
2. **Only one 88305 code may be posted for each separate specimen.** The same principle applies to cell blocks as to other preparation methods: if multiple cell blocks are prepared for a specimen, you should report 88305 only once.

3. **Each histochemical and immunohistochemical study is charged per stain or antibody used.** Special stain codes 88312 and 88313 and immunohistochemical stain code 88342 are charged and reported for each different stain or antibody used per specimen (e.g., Gomori methenamine silver (GMS) and periodic acid-Schiff [PAS] on one sample constitute two separate charges).
4. **Each preparation method, special stain, and immunohistochemical antibody that was used must be named in the report for proper billing.** Third-party payer auditors assume that billed procedures were not actually performed if they are not properly documented in the medical report; unsupported charges are denied, and additional sanctions can be imposed if the auditor deems the problem to be egregious.

Coding Fine-Needle Aspirates

Like the rules for non-gynecologic or non-FNA specimens, the coding rules for FNAs are relatively straightforward. When coding an FNA, the specimen type does not play a role. It does not matter, for example, if the FNA was from the breast, thyroid, or salivary gland. What matters is what procedures were used to obtain and examine the specimen. The procedure (charge) codes for coding FNAs are given in [Table 17.5](#). There are four basic codes: extraction by a pathologist without image guidance (**10021**), extraction by a pathologist with image guidance (**10022**), immediate study for sample adequacy (**88172**), and diagnosis (**88173**).

Several points regarding billing for FNAs deserve emphasis.

1. **The fundamental unit for billing the extraction codes (10021, 10022) and the diagnosis code (88173) is the specimen, not the “pass.”** Only one extraction code (10021 or 10022) may be billed per

TABLE 17.5 PROCEDURE CODES FOR FINE-NEEDLE ASPIRATIONS

Procedure	Code
Extraction by a pathologist without image guidance	10021
Extraction by pathologist with image guidance	10022*
Immediate study (adequacy assessment)	88172
Diagnosis	88173
“Add-on” procedures:	
-cell block	88305
-special stains for microorganisms	88312
-other histochemical stains (e.g., periodic acid-Schiff)	88313
-immunohistochemical stains	88342

*A radiology needle placement code may also be billable.

lesion or anatomic site, even though it involved multiple passes. Similarly, only one diagnosis code 88173 may be billed per specimen, even when each pass from one lesion is received in a separate vial. If separate anatomic sites are aspirated (e.g., left and right neck masses or distinct nodules from the right neck), then separate 10021 (or 10022) charges may be billed for each site, and separate diagnosis charges (88173) are warranted, too.

2. **To bill codes 10021 or 10022, the report must state that the pathologist personally extracted the specimen.** An extraction code may be charged for a procedure performed by a resident or fellow so long as the senior pathologist is physically present in the procedure or operating suite to personally supervise the procedure. A pathologist cannot bill 10021 for simply assisting another physician (e.g., radiologist, surgeon) who performs the FNA.
3. **The fundamental unit for billing an immediate assessment (88172) is the “pass.”** More than one immediate assessment code (88172) may be billed if more than one pass was needed to obtain an adequate specimen. If you choose to bill for each separate immediate study, the result of your assessment of each pass must be documented in the report. For example, if adequacy (or a diagnosis) was established by the third pass, it is not appropriate to bill more than three 88172 charges, even if more passes were obtained. A word of caution: you may not want to report more than one 88172 when you are the physician performing the FNA. Such self-imposed conservatism is a good idea because there may be a perceived conflict of (financial) interest when you are assessing adequacy and also performing the FNA.
4. **The “immediate study” may be limited to an assessment of adequacy.** The immediate study does not need to be a diagnosis; it may involve just an adequacy assessment. Code 88172 applies in either case.
5. **The facility (e.g., hospital) may also report 88172.** The resources of the facility (e.g., microscope, cart, slides, stain, room depreciation, and interest) are being used to support the work of the pathologist. Hence, if the pathologist is entitled to report 88172, the hospital or independent lab that supports the procedure is justified in reporting that code too, including multiple units of the code when warranted.
6. **Code 88172 applies for any immediate study, no matter who performed the FNA.** It does not matter if the extraction was performed by a pathologist, surgeon, or radiologist.

7. **Code 88172 is appropriate for cases in which the slides are “rushed” to the cytology laboratory while the patient is kept on the radiology table, not just those performed in the radiology suite.** Once the patient leaves the procedure room, however, there is no longer any value to an immediate assessment, and 88172 should not be reported if the immediate study is performed after that point.
8. **The CAP advises that code 88172 is not reportable if a CT alone performs the immediate study.** The CAP offers no rational explanation for why you may not bill for the services of a CT to perform adequacy readings on FNAs, even though it is within their scope of practice and commonly done. (The efficacy of CAP’s advice in these regards is hotly contested at the time of this writing, so you should consult with your compliance advisor before establishing a charge policy covering this situation.)
9. **Do not report interpretation code 88173 if an FNA is completely acellular.** This includes “blood only” samples, when the apparent cause is technique-based. On the other hand, if the sample is scanty but some cellular elements were identified and examined, it is appropriate to report 88173. Be careful when using the word *nondiagnostic* in the report. Payers will usually deny the charge when they see that word because it connotes, in layman terms, the absence of medical necessity. You can minimize disputes in such cases by elaborating on your findings in the report with phrases like “occasional histiocytes present,” or “fragments of skeletal muscle and bone.”
10. **According to the CAP, codes 88108 and 88112 may not be reported in addition to code 88173 for the same FNA specimen.** According to the CAP, 88173 covers all slide preparations for an FNA, excluding only cell block slides. This advice is contrary to conventional wisdom. You will remember from the previous discussion that the AMA allows reporting 88104 and 88108 together for a nongynecologic or non-FNA specimen. It seems illogical to permit multiple preparation code billing for nongynecologic specimens but not for FNAs. Medicare, it is true, does not permit reporting 88108 or 88112 in addition to 88173. For non-Medicare payers that do not explicitly abide by Medicare CPT rules, you must decide (in consultation with legal counsel and your compliance officer) whether you will report 88108 or 88112 with 88173.
11. **The “extended cytology study” code (88162) never applies to FNA specimens.** No matter how many slides are prepared or how many different routine stains (Papanicolaou and Wright-Giemsa) are used, do not report 88162 with an FNA specimen, either as an add-on code or instead of 88173.

12. In the event that only concentrated (e.g., cytospin) or enriched or concentrated (e.g., ThinPrep®) preparations are made (no smears), the “base” code 88173 is retained. Code 88173 applies to an FNA specimen, regardless of the base preparation. An add-on code (88108 or 88112) is not used under such circumstances.
13. **As with nongynecologic, non-FNA specimens, special stains and immunohistochemical stains are charged per stain or antibody used, and each must be named in the report.** A cell block (88305) is also separately chargeable, following the same rules as were mentioned previously.

Additional codes are available for charging what are called **Evaluation and Management (E/M) services** in conjunction with an FNA. Several preconditions must apply to justify these additional codes. The referring physician must explicitly request such a consultation; the pathologist must obtain a focused clinical history and perform an appropriate physical examination; and the cytology report must go beyond the usual FNA report by including a consultation summary. A detailed discussion of E/M codes as they relate to FNAs is beyond the scope of this chapter, but it is important for the reader to know that they are available and applicable to pathologist-performed FNAs.

Coding Consultation Cases

In pathology, accurate diagnosis sometimes rests on the input of a consultant who is especially knowledgeable in a particular area. Payers and regulators acknowledge that, under certain circumstances, this is a medically justified activity and should be reimbursed. The following three codes are used for billing a consultation service. These apply to surgical pathology and cytopathology consultations.

Codes for consultation on outside slides or materials:

- 88321 Outside slides
- 88323 Outside slides + in-house hematoxylin-eosin (H & E) slide preparation
- 88325 Outside slides + “much more”

Code 88321 applies when you do not need to make any *routine* preparations (e.g., H & E) in addition to the slides prepared elsewhere. This code accounts for the majority of pathology or cytology consultations. It applies whether you are reviewing just one slide or many more, including special stains and immunohistochemistry preparations. It also applies if you need to make additional *nonroutine* preparations (special stains, immunohistochemistry), but not an additional routine slide.

Code 88323 is used when a consultation case requires additional routine (e.g., H & E) slide preparation, usually from a submitted paraffin block. A slide prepared primarily for the consultant's file does not warrant an upgrade from 88321 to 88323.

Code 88325 is known as the “comprehensive” consultation code and applies when a consultation requires the review of medical records and other materials (e.g., clinical lab results, oncologist's report) beyond the pathology report and slides. This code seldom applies to cytopathology consultation cases.

Several points regarding billing for consultations deserve emphasis.

1. **A consultation does not need to be initiated by an outside pathologist.** A patient may be coming to see another physician at your hospital, and that physician may request that you review the outside materials.
2. **Standard add-on procedures** for special stains and immunohistochemistry are separately chargeable when ordered by the consultant from his or her lab, if medically indicated.
3. **A consultation fee cannot be charged for intra-group consultations.**
4. **You cannot consult with yourself by charging an extra consultation fee for reviewing prior materials on a patient whose current specimen you are examining.** The consultation ordinarily must be initiated by a physician unrelated to your practice.
5. **You must generate some sort of report to bill a consultation charge.** This can be a formal cytology report or a letter to the outside pathologist.
6. **The basic (charge) unit of service for consultation cases is “each case.”** Often, multiple specimens from a patient are received for consultation (e.g., five Pap tests over a 7 year-period). According to AMA rules, you are entitled to report 88321 five times for reviewing these cases, even if your review is resulted as a single consultation report (as is commonly done) that lists all five cases. If, on the other hand, you receive three specimens that were part of a single outside case (e.g., S08-1234A, S08-1234B, and S08-1234C), then you are entitled to bill only one consultation code. Notwithstanding the AMA's advice, effective Oct. 1, 2007, Medicare permits you to bill only one unit of one consultation code per beneficiary per date of consultation. The various permutations that govern this rule can get rather complex, and a complete discussion is beyond the scope of this chapter.

As a result of Medicare's 2007 change in coverage policy for consultation cases, at the time of this writing the profession was in the process of reexamining the basic consultation charge parameters previously described. Fundamental changes to consultation billing policies may have occurred since that time. Indeed, significant billing

policy changes can occur at any time. It is thus important to periodically consult with your coding advisor to determine the latest accepted charge coding rules.

QUALITY CONTROL AND QUALITY ASSURANCE

All tests have inherent limitations, and cytology is no exception. Even in the hands of well-trained and conscientious physicians and cytologists, false-negative and false-positive results can happen. Potential sources of error are particularly well understood when it comes to Pap testing.

False-negative Pap interpretations result from:

- “sampling error”
 - the collection device does not sample lesional cells, or
 - there is inefficient transfer of lesional cells from the collection device to the glass slide¹²
- laboratory error
 - screening (i.e., abnormal cells are missed)
 - interpretation (i.e., abnormal cells are misinterpreted as benign)

The CLIA 88 Final Rule established QC standards to minimize the laboratory component of such errors. Some of the QC standards have been reviewed previously. In addition, three forms of slide reexamination are required in the United States, all related to gynecologic specimens.

Cytology slide reexamination requirements:

- prospective rescreeing of 10% of negative Pap cases (“10% rescreen”)
- retrospective review of all negative Paps from women with a newly diagnosed high-grade squamous intraepithelial lesion (HSIL; “5-year lookback”)
- review of discrepancies between Pap and biopsy results.

Each of these is discussed in turn in the sections that follow. Documenting compliance with and generating statistics from QC activities is greatly aided if one has robust cytology software programs within one’s laboratory information system.

Prospective 10% Rescreen

The prospective rescreeing of 10% of negative Paps has been a QC requirement of all laboratories in the United States since the 1960s, predating CLIA 88 but reaffirmed

by it.¹³ Some of the negative Pap cases for review must be selected randomly and others by targeting high-risk women, most commonly those with a history of a squamous intraepithelial lesion (SIL). The laboratory must specify how the cases are selected at random and how cases from high-risk women are identified. The rescreeing must be carried out prospectively, so that errors in diagnosis can be corrected before the report is issued. The rescreeing must be done by a TS, a GS, or a CT with 3 years of full-time experience in the past 10 years.

There is one exception: for laboratories with a solo pathologist and no CT, this rescreen need not be performed.

The rationale for this requirement is to identify CTs whose work is unreliable. The drawback is that, given the prevalence of disease in most populations, it could take more than 10 years using this method to identify a CT with a high error rate if the threshold for an error is set at SIL.¹⁴ The percentage of rescreeed cases upgraded to SIL or invasive cancer ranges from 0.2% to 5%.¹⁵⁻¹⁷ Even when defined as an upgrade to atypical squamous cells (ASC) or worse, some have reported a frequency as low as 0.18%.¹⁸ These calculations, whereby the number of errors is divided by the number of slides rescreeed, provides only a crude error “rate” that does not control for the prevalence of disease in a population. The strictly defined “false-negative rate” (FNR) is the proportion of *abnormal Paps* that are falsely called negative. The FNR and its use in evaluating performance will be discussed later in the chapter.

Retrospective Rescreen (“5-Year Lookback”)

Retrospective rescreeing, which targets archived negative Paps from women with a new Pap interpretation of HSIL or cancer, was introduced with CLIA 88. By rescreeing all previous negative Paps during the 5-year period before a new Pap diagnosis of HSIL or cancer, the likelihood of detecting an error is greater than with prospective rescreeing of negative Paps. The percentage of diagnoses reclassified as unsatisfactory (UNS), ASC, or worse ranges from 12% to 94%¹⁹⁻²⁶ (Table 17.6). Not surprisingly, the higher the intensity of rescreeing, the greater the likelihood of detecting an abnormality on review.²¹ The estimated frequency of errors in many published reports is inflated, however, because the Paps were reviewed with the knowledge that all the women had a current HSIL or worse Pap result. When controlled for retrospective bias, the percentage of cases reclassified is lower.²³

Some investigators feel that a 2-year retrospective review, which identifies 75% of all false negatives (FNs), is sufficient for QC purposes.¹⁹ Others have found that a 3-year review identifies 94% of all undercalls.²⁵

TABLE 17.6 RESULTS OF 5-YEAR RETROSPECTIVE RESCREENING

Study	Number of Slides Rescreened	Percentage of Cases Reclassified as ASC or Worse (Including UNS)
Allen et al. ^a	80	18
Gatscha et al. ^b	422	29
Hatem and Wilbur ^{*c}	17	94
Nick et al. ^d	351	71
Jones ^e	3762	20
Tabbara et al. ^{ft}	58	38
Montes et al. ^{g†}	100	12

*Only 2 years reviewed.

[†]Rescreened seven cases originally called atypical squamous cells, undetermined significance (ASCUS) or atypical glandular cells, undetermined significance (AGCUS). The ASCUS and AGCUS categories were not used for reclassification.

[‡]Included controls for retrospective bias.

^aAllen KA, Zaleski S, Cohen MB: Review of negative Papanicolaou tests: Is the retrospective 5-year review necessary? *Am J Clin Pathol* 1994;101:19-21.

^bGatscha RM, Warren GP, Saigo PE: Quality assurance: insight into a laboratory's performance [Abstract]. *Lab Med* 1994;25:258.

^cHatem F, Wilbur DC: High grade squamous cervical lesions following negative Papanicolaou smears: False-negative cervical cytology or rapid progression. *Diagn Cytopathol* 1995;12:135-141.

^dNick TG, Bush DF, Cason Z, Lemos LB: Review of negative Pap smears: Quality assurance protocol for high-grade squamous intraepithelial lesions [Abstract]. *Acta Cytol* 1995;39:90.

^eJones BA: Rescreening gynecologic cytology: Rescreening 3762 previous cases for current high-grade squamous intraepithelial lesions and carcinoma—a College of American Pathologists Q-Probe study of 312 institutions. *Arch Pathol Lab Med* 1995;119:1097-1103.

^fTabbara SO, Sidawy MK: Evaluation of the 5-year review of negative cervical smears in patients with high grade squamous intraepithelial lesions. *Diagn Cytopathol* 1996;15:7-11.

^gMontes MA, Cibas ES, DiNisco SA, Lee KR: Cytologic characteristics of abnormal cells in prior "normal" cervical/vaginal Papanicolaou smears from women with a high grade squamous intraepithelial lesion. *Cancer (Cancer Cytopathol)* 1999;87:56-59.

ASC, atypical squamous cells; UNS, unsatisfactory.

If a significant discrepancy is found that will affect current patient care, the patient's physician must be notified and an amended report issued. Such instances are vanishingly rare because the review is prompted by a current abnormality at the level of HSIL or higher.

Cytologic-Histologic Correlation

Cytologic-histologic correlation is the mainstay of QC in cytology, the foundation for developing and refining cytologic diagnostic criteria. Histologic outcome is a common gold standard against which cytologic interpretations, gynecologic and nongynecologic, are measured.

It is important to recognize, however, that perfect cytologic-histologic correlation is not realistic. Discrepancies between Paps and biopsies, in particular, are not uncommon (range, 11% to 32%; [Table 17.7](#)). The majority of discrepancies are the result of so-called "sampling" error,^{17,27-30} implicated when a review of the discrepant Pap-biopsy pair confirms both original diagnoses. Sometimes biopsies are negative because colposcopy has an inherent FNR (i.e., the lesion is simply not detected colposcopically). The ASCUS/LSIL Triage Study (ALTS) found that 33% to 36% of histologic cervical intraepithelial neoplasia (CIN) 2 and worse lesions were missed by colposcopy.^{31,32} Even when detected colposcopically and biopsied, sometimes the lesion is not properly embedded in paraffin or adequately sectioned. Errors in cytologic and histologic interpretation do occur, however, but are less common than sampling errors.

The positive predictive value (PPV) of the Pap test is directly proportional to the degree of the Pap abnormality. Women with a Pap diagnosis of ASC have a 44% to 62% chance of having SIL at colposcopy.³³⁻³⁵ For a Pap diagnosis of low-grade SIL (LSIL), the PPV for SIL is 50% to 86%.^{33,35-38} When the biopsy from a woman with an LSIL Pap shows a lesion, it is usually low grade, but in 12% to 17% it is high grade.^{35,37,38} For a Pap diagnosis of high-grade SIL (HSIL), the PPV for SIL is around 82%.³⁵

A discordance in lesion grade accounts for approximately 43% of discrepant Pap-biopsy interpretations.³⁹ The woman with an HSIL Pap but an LSIL biopsy is a particular dilemma for the physician, who almost always relies on the biopsy to guide management. Nevertheless, data suggest that the HSIL Pap interpretation in such cases cannot be entirely discounted: there is a higher prevalence of high-risk human papillomavirus (HPV) types in these women, and a greater risk of HSIL on follow-up.³⁹

CLIA 88 requires that laboratories compare Pap and cervical biopsy reports and determine the cause of any discrepancies in interpretation.¹³ At a minimum, CLIA 88 requires that all Paps reported as HSIL, adenocarcinoma, or other malignant neoplasms be correlated with subsequent histopathologic reports.² The way in which Pap-biopsy discrepancies are reviewed, however, varies from laboratory to laboratory. The variables include (1) the time interval between the Pap and the biopsy that defines case inclusion; (2) the definition of a discrepancy; (3) the timing of the review (e.g., during or after the biopsy signout); and (4) the individuals assigned responsibility

TABLE 17.7 ANALYSES OF DISCREPANCIES BETWEEN PAPS AND BIOPSIES

	Joste et al. ^a	Tritz et al. ^b	Rohr ^c	Ibrahim et al. ^{d†}	Jones and Novis ^e
Frequency of a discrepancy (%)	11	11	32	14	16
Number of discrepancies reviewed	175	69	104	481	2971
Reasons for discrepancy					
Sampling error [‡] (%)	93	61	75	93	86
Pap errors					
Screening (%)	0.6	2.9	4.8	4	2
Interpretation (%)	2.8	11	15	1.6	8
Biopsy errors					
Interpretation (%)	5.7	13	3.8	1.6	8
Histochemical processing (%)	0	4.3	0	0.2	0
Combination (%)	0	7.2	0	0	0

[†]Compared only concurrent Pap-biopsy pairs.

[‡]Sampling error includes biopsy and cytologic sampling.

^aJoste NE, Crum CP, Cibas ES: Cytologic/histologic correlation for quality control in cervicovaginal cytology: Experience with 1,582 paired cases. Am J Clin Pathol 1995;103:32-34.

^bTritz DM, Weeks JA, Spires SE, et al.: Etiologies for non-correlating cervical cytologies and biopsies. Am J Clin Pathol 1995;103:594-597.

^cRohr LR: Quality assurance in gynecologic cytology: What is practical? Am J Clin Pathol 1990;94:754-758.

^dIbrahim SN, Krigman HR, Coogan AC, et al.: Prospective correlation of cervicovaginal cytologic and histologic specimens. Am J Clin Pathol 1996;106:319-324.

^eJones BA, Novis DA: Cervical biopsy-cytology correlation. A College of American Pathologists Q-Probes study of 22,439 correlations in 348 laboratories. Arch Pathol Lab Med 1996;120:523-531.

for discrepancy resolution. Some laboratories choose to review only concurrent Paps and biopsies²⁸; others include Paps that precede a biopsy, provided the Pap was obtained within a 3-month period before the biopsy.³³ Some laboratories consider an ASC Pap discordant if the biopsy shows SIL²⁸; others do not.⁴⁰ In some laboratories, all discrepant Pap-biopsy pairs are reviewed by the CP responsible for the current biopsy interpretation. In others, the responsibility falls on the CP who made the original Pap interpretation.²⁷ In yet others, all Paps and biopsies are reviewed independently by several CPs, and the review interpretation is an average of several interpretations.^{17,40} Some laboratories perform discrepancy resolution at a weekly consensus conference.³⁰ Having a choice among a variety of discrepancy resolution methods allows a laboratory to select the one that fits its workflow best. Some of the variables, and how laboratories handle them, are summarized in a CAP Q-Probes study.³³

Statistics

The CLIA 88 regulations require that cytology laboratories compile annual statistics.

Annual statistics must document:

- the number of cytology cases examined
- the number of specimens by specimen type (e.g., urine, sputum, etc.)
- the volume of cases by diagnosis (e.g., negative, atypical, suspicious, positive)
- the number of unsatisfactory cases

- the number of Pap tests with discrepant histologic results
- the number of negative Pap tests that were reclassified as abnormal
- the number of Paps reported as HSIL, adenocarcinoma, or other malignant neoplasm with no histologic follow-up

Workload Records

The CLIA 88 regulations established, for the first time, workload limits for CTs in the United States. The maximum number of slides is 100 per 24-hour period. This applies to anyone who does primary screening, whether CP or CT. The minimum amount of time spent examining this maximum is 8 hours (average 12.5 slides per hour). If a CT spends less than 8 hours screening, the maximum number of slides is prorated using the formula: number of hours examining slides $\times 100/8$.

The maximum of 100 slides per 24 hours is not intended as a performance target, but rather as an absolute maximum allowed by law.

Each CT is responsible for keeping records of the total number of slides examined each day and the number of hours spent screening. If a CT works at one or more other laboratories that day, the hours spent working at the other laboratories and the number of slides screened there must also be recorded and taken into account by the TS of the primary laboratory, to ensure that the total number of slides at all laboratories does not exceed the maximum allowed.

The limit on slides includes gynecologic and nongynecologic cases, and slides rescreened for QC purposes. Not all slides are created equal. Smears are counted as one slide, but nongynecologic slides in which the cellular material covers one half or less of the slide surface are counted as a half slide.

Slides that typically count as half slides:

- cytocentrifuge preparations
- cell block sections
- ThinPrep slides (applies only to nongynecologic cases; gynecologic cases count as a full slide)²
- SurePath slides (applies only to nongynecologic cases; gynecologic cases count as a full slide)²

According to CLIA 88, when performing evaluations using automated or semi-automated screening devices, the calculations of slide equivalencies must follow the manufacturer's instructions.

The 100 slides/day rule is an absolute maximum, but the actual workload limit is set by the TS based on performance evaluations. The actual workload limit may be 100 slides/day, or it may be lower, but there must be evidence that the performance of each CT is evaluated every 6 months and that the limit is established in accordance with performance measures.

Custom-designed or off-the-shelf software within one's laboratory information system (LIS) is the easiest and most accurate way to document compliance with workload limits. A good LIS can automatically track the number of slides examined when the CT enters his or her provisional (or final, in the case of negative Paps) interpretation. The CT need only enter the number of hours spent screening, and the number of slides screened and time spent screening at another laboratory, if applicable, for the LIS to calculate the daily workload.

PROFICIENCY TESTING

Despite provisions in CLIA 88 for cytology proficiency testing (PT), a national cytology PT program was not approved until 2004. (CMS had approved an examination offered by the **State of Maryland**, but this test was only available to practitioners in that state.) The first vendor to obtain approval by CMS to offer a national PT program was the **Midwest Institute for Medical Education (MIME)**. Approval of the MIME program was granted in the fall of 2004 for testing in 2005. A year later, in September of 2005, CMS approved the **CAP's** Pap PT program for testing beginning in 2006. In 2006, the **American Society for Clinical Pathology (ASCP)** acquired the complete cytology product line of MIME, including its PT program. For 2005, 2006, and 2007, CMS decided that laboratories and individuals would have

no sanctions or deficiencies applied against them or their CLIA certificate if individuals failed the test, provided all individuals and laboratories were enrolled in a CMS-approved testing program. In other words, the PT programs in those years operated with an emphasis on education.

General description of cytology PT:

1. The test is administered on site every year. Under most circumstances, it is an announced test that has been scheduled at least 30 days in advance.
2. Each participant evaluates 10 gynecologic slides.
3. Each participant is allowed 2 hours to complete the test.
4. Participants are allowed to select the type of Pap preparation (smear, ThinPrep, SurePath) they are most accustomed to.
5. Pathologists who ordinarily examine Pap slides after they are prescreened by a CT may choose to screen a set of test slides that have been previously screened and dotted by a CT. If the pathologist chooses to examine a prescreened set, the CT's diagnosis accompanies the test set.
6. Participants are proctored during the 2-hour period.
7. The laboratory documents the slide handling, proctoring, and testing process.
8. A copy of all records is maintained by the Laboratory Director.

Scoring:

There are only four possible answers ("response categories") to each test case:

- a. Unsatisfactory for diagnosis.
- b. Negative. (This may include infectious organisms or inflammatory or reactive changes, but not HPV effect.)
- c. LSIL.
- d. HSIL or cancer.

Each test set must contain at least one slide from each of these four response categories.

The scoring grid is different for cytotechnologists ([Table 17.8](#)) versus cytopathologists ([Table 17.9](#))

Results:

Once cytology PT is fully functional, individuals face specified sanctions if they do not pass the examination. To pass, a participant must attain an overall score of 90% or higher. An individual who passes the test does not need to be tested until the following year.

If a passing score of 90% is not achieved on the initial test, the participant is allowed to take a second 10-slide test within 45 days after notification of test failure. When an individual passes the second 10-slide test, he or she has successfully participated for the year and need not be tested again until the following year. If the participant fails the retest (second event):

- The individual must obtain documented, remedial training in the area of test failure. (The "area of test failure" is noted on the test results letter).

TABLE 17.8 SCORING GRID FOR CYTOTECHNOLOGISTS

Correct Response	Participant Response			
	A. UNSAT	B. NEG	C. LSIL	D. HSIL
A. UNSAT	10	0	5	5
B. NEG	5	10	5	5
C. LSIL	5	0	10	10
D. HISL/CA	0	-5	10	10

CA, cancer; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NEG, negative; UNSAT, unsatisfactory.

TABLE 17.9 SCORING GRID FOR CYTOPATHOLOGISTS

Correct Response	Participant Response			
	A. UNSAT	B. NEG	C. LSIL	D. HSIL
A. UNSAT	10	0	0	0
B. NEG	5	10	0	0
C. LSIL	5	0	10	5
D. HISL/CA	0	-5	5	10

CA, cancer; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NEG, negative; UNSAT, unsatisfactory.

- All Pap slides screened by the individual after notification of failure must be reevaluated.
- The individual must successfully participate in a 4-hour, 20-slide test.

If the individual fails the third testing event (20-slide test):

- The individual must cease examining Pap slides immediately on notification of failure.
- The individual must obtain at least 35 hours of documented, formally structured, continuing education in diagnostic cytopathology which focuses on the examination of gynecologic cytology specimens.
- The individual must successfully pass a 20-slide proficiency test.

This final cycle would continue until the individual successfully participates in a 20-slide proficiency test.

Information on aggregate annual pass-fail rates can be found on the CMS Web site at www.cms.hhs.gov/CLIA/02_CytologyProficiencyTesting.asp#TopOfPage.

In 2005, a substantial difference was found in the first-test failure rates of pathologists with CTs (10%) versus those without CTs (33%).

PERFORMANCE EVALUATION

Objective measures of CT and CP performance can be distilled from the CLIA-mandated QC activities

previously described. In many cases, the data are not absolute measures of accuracy, but rather allow comparisons of individuals to their peers, reflected in the laboratory average. The TS can then identify outliers who may need educational enhancement or an adjustment in their workload limit. As mentioned, the TS has responsibility for evaluating the performance of all CTs every 6 months and determining the actual workload limit of each CT for the next 6-month period. According to CLIA 88, the TS's assessment of the CT must be based on (1) results from the 10% rescreen of negative Paps and (2) level of pathologist concordance with the CT's interpretations on the cases referred for CP review.

Performance evaluation is more problematic in small laboratories with only one or two CTs or CPs because an intralaboratory comparison of the CT to his or her peers is not feasible. In this setting, competency can be evaluated in part by using commercially available, glass slide-based gynecologic and nongynecologic testing modules available from professional societies like CAP and ASCP. Results from many laboratories are tabulated, allowing comparison with one's peers across the country. Information on these programs can be found at the CAP and ASCP Web sites.

Although CLIA 88 does not explicitly require performance evaluation of CPs, the Joint Commission does require periodic (biannual) recredentialing of all physicians, which is based on an assessment of performance.

Measures of Cytotechnologist Performance

A TS works closely with the CTs, and particularly through daily case review of referred Paps and nongynecologic specimens, he or she develops a subjective impression of their competency. These subjective impressions are valuable, but it is wise to temper them with objective measures of performance.

Rationale for objective measures of performance:

- reinforce subjective impressions
- rectify misperceptions
- carry more weight with regulatory agencies
- may be more acceptable to the employee than subjective evaluations as justification for educational enhancement

A CT's role in slide review can be broadly divided into two functions, **screening**, or the detection of significant cells, and **interpretation** of cellular findings, with documentation of a provisional (in the case of abnormal Paps and all nongynecologic cases) or final (in the case of negative Paps) interpretation. Both skills should

be evaluated. Objective measures of a CT's performance are tabulated at periodic intervals (e.g., monthly or every 6 months).

Screening Skills

A CT's screening skills can be assessed from (a) the FNs detected during the rescreeing of 10% of negative Paps, and (b) the percentage of cases called abnormal ("abnormal rate") by the CT.

False Negatives. Inevitably, when enough cases interpreted as negative by one CT are rescreened by another, abnormal cells are identified in some of them. Even the most conscientious, well-trained, and alert CT eventually misses abnormal cells. These cases are called false-negatives. The prospective 10% rescreen, originally enacted in the 1960s, was intended as a tool for identifying CTs with less-than-optimal performance. As mentioned, the 10% rescreen is not particularly effective in documenting performance because the number of missed SILs, at least over a 6-month period, is low and does not allow meaningful comparison among CTs.¹⁴ For this reason, when the CLIA 88 regulations were submitted for public comment, many argued for abolishing this QC requirement.¹³ It was nevertheless retained, and most laboratories continue to monitor FNs for performance evaluation. In fact, CLIA regulations now require that performance be evaluated using results of the 10% rescreen.²

The 10% rescreeing requirement has some merit; it puts CTs on notice that each Pap slide they screen has a chance (at least 1 in 10) of being reviewed by another CT.

FNs are truly uncommon when the threshold for an FN is set at SIL. An editorial published in 1994 suggested lowering the threshold to ASC.⁴¹ At this lower threshold, the authors argued, errors are detected frequently enough to make statistically meaningful comparisons among CTs. This argument has merits and drawbacks. Even with a lower threshold, errors may not be frequent enough for statistical significance.^{18,42} The argument is also predicated on the assumption that ASC is a reproducible diagnosis, a tenuous thesis at best.⁴³⁻⁴⁵ To improve statistical significance, at least, the definition of an "error" could be expanded even further to include an incorrect statement of adequacy or a missed infectious agent.⁴⁶

In addition to tallying FNs as raw numbers per CT over time, a statistically useful FNR (also known as the "FN fraction" or "FN proportion") can be calculated. The FNR is defined as the proportion of all *abnormal Paps* that are falsely negative.

Thus, the

$$\text{FNR} = \text{FN} / (\text{TP} + \text{FN}),$$

where TP is "true positives." A sample calculation is given in Table 17.10. In this example, 5% of negative Paps were rescreened at random. (Another 5% were rescreened from the high-risk pool.) Not all interpre-

TABLE 17.10 SAMPLE FALSE-NEGATIVE RATE CALCULATION

Total Paps screened	10,000
Abnormal Paps diagnosed	500
5% random rescreen	4 FNs
Estimated total FNs	$4 \times 20 = 80$
FNR	Total estimated FNs/total abnormals $= 80 / (500 + 80) = 14\%$

FN, false-negative; FNR, false-negative rate.

tation errors are detected if only 5% of negative Paps are randomly rescreened. Therefore, a total "estimated FNs" must be calculated by multiplying the number of FNs detected (4 in this example) by 20, thereby extrapolating to a 100% rescreeing effort (i.e., if 100% of the negative Paps had been rescreened, 80 FNs would have been detected).

FNRs in published literature range from 0% to 28%.^{41,47} FNRs can be calculated for individual CTs and used for performance evaluation, particularly if the LIS is programmed to do this calculation.⁴⁸ The challenge is to establish benchmarks and action thresholds. Although there are no national benchmarks,⁴² a laboratory director who uses FNRs for CT performance evaluation can set a threshold for performance and define followup action when this threshold is crossed.⁴⁷

Note that FNRs cannot be used to compare one laboratory to another because the rescreeing effort may vary in its thoroughness from one laboratory to another. Ironically, a laboratory that does a more thorough job of rescreeing will have a higher FNR than one that is less thorough. An FNR that corrects for the accuracy of the rescreeing process is a better measure of the accuracy of a laboratory,⁴⁹ but this method requires effort and is not widely applied.

Data generated from the retrospective rescreen ("5-year lookback") are difficult to apply to performance evaluation. Among other reasons, many of the errors detected were made 4 or 5 years previously, and it is difficult to justify evaluating someone's current performance based on errors made several years earlier.⁸

Abnormal Rate. The abnormal rate is the percentage of abnormal cases (ASC, AGC, SIL, and carcinoma) diagnosed by a CT divided by the total number of cases examined. The abnormal rate of each CT is useful for performance evaluation because it allows comparison with the laboratory average. A lower than average abnormal rate suggests that significant lesions are being missed and can be used as a double-check against the FNR, another measure of screening competency. In fact, CLIA 88 requires that the "case reviews" of individual CTs be evaluated against the laboratory's annual statistics and that discrepancies be documented and addressed.

The significance of any individual CT's variance from the lab average can be measured using statistical

measures of variance like the Z score, also called the standard normal deviate.⁵⁰ A Z score greater than 2.0 shows an abnormal rate more than two standard deviations (SD) above the average. More importantly, a Z score less than -2.0 indicates an abnormal rate two SDs lower than the lab average, suggesting that there may be a problem in detecting abnormals.

Note that statistical comparisons are reliable only if there is an equal distribution of abnormals in the cases screened by the CTs.

Interpretive Skills

Cytotechnologist/Cytopathologist Discrepancy Log.

CLIA 88 requires that the performance of a CT be based, in part, on an evaluation of the cases submitted to the pathologist for review. In addition to the valuable subjective impressions obtained from the daily interchange between CT and CP, performance can be evaluated using quantitative measures of the degree of agreement.^{8,51} A well-designed LIS permits easy tracking of concordance rates. Software programs can tabulate the frequency and severity of discordance and provide statistical measures such as κ values. The κ value, a statistical measure of the degree of concordance between two observers, here the CT and CP, ranges from 0 to 1.0, with 0 being chance agreement and 1.0 being perfect concordance.⁵² Monitoring such measures is useful in identifying CTs with diagnostic interpretative difficulties.⁵¹

Unsatisfactory Rate. The unsatisfactory rate is the proportion of all Paps that are interpreted as unsatisfactory by a CT. A low unsatisfactory rate suggests that insufficiently stringent adequacy criteria are being applied. As with the abnormal rate, calculating the significance of any variance from the laboratory average can be helpful.

Measures of Cytopathologist Performance

Atypical Squamous Cell-to-Squamous Intraepithelial Lesion Ratio

Because ASC is essentially a diagnosis of uncertainty, it is reasonable, as a QC measure, for a laboratory to monitor its ASC/SIL ratio and that of its individual CPs to see if it (or any individual CP) may be using the ASC interpretation too frequently. To this end, useful benchmarks exist: a proposed upper limit (3:1)⁵³ and nationwide percentile rankings for ASC:SIL ratios based on large surveys of laboratories compiled by the CAP. Periodic confidential feedback to pathologists on their ASC/SIL ratio can have a beneficial effect, particularly on outliers.^{54,55}

The ASC/SIL ratio is a useful measure of CT and CP performance. As a measure of CT performance, the ratio

must be calculated from the CT's provisional interpretation, not the final one that was resulted by the CP.

High-Risk Human Papillomavirus-Positivity Rates for Atypical Squamous Cells of Undetermined Significance

Another performance measure of CPs is based on the commonly used "reflex" test for high-risk human papillomavirus (HR HPV) in women with a cytologic interpretation of ASC-US.⁴³ A laboratory and, by extension, an individual pathologist, can assess their aggregate ASC-US interpretations over time by seeing if the frequency of positive HR HPV test results conform with accepted benchmarks like those from the ALTS trial (50.6%).⁵⁶ In this manner HPV test results are, in essence, used to adjudicate aggregate morphologic interpretations.⁵⁷⁻⁵⁹

There is little correlation between the ASC/SIL ratio and the percentage of positive HR HPV test results for ASC-US interpretations.⁵⁷ The lack of correlation is not surprising because these two variables measure different aspects of a CP's performance. The ASC/SIL ratio is a measure of the degree of uncertainty in the interpretation of cytologic findings, expressed as a ratio of the number of diagnoses of uncertainty (ASC) to a number of diagnoses of certainty (SIL). By contrast, the percentage of ASC-US cases that are positive for HR HPV DNA is an objective assessment of the risk of dysplasia in an aggregate population of ASC-US cases because aggregate ASC-US cases are measured against an external standard, namely, the presence or absence of an oncogenic virus. Importantly, a benchmark for the HR HPV-positive rate of ASC-US cases adjudicated by experienced CPs exists in the ALTS trial database.⁶⁰

Drift from the desired norm, in practice, comes in a variety of combinations of an ASC/SIL ratio (low, average, high) and an HR HPV-positive rate for ASC-US (low, average, high).⁵⁹ It is important for the laboratory director and the CP to understand the underlying forces that can cause indicators to shift from the norm. A working schema that explains the possible alterations from the desired ASC/SIL ratio or HR HPV-positive rate for ASC-US can be useful for understanding the forces that perturb these indicators.⁵⁹ Identifying such patterns and finding an explanation for perturbations in one or both measures can be useful to the CP, who can make an informed decision to adjust a diagnostic threshold and monitor the result.

It is important to note that neither the ASC/SIL ratio nor the HR HPV-positive rate for ASC-US is a measure of diagnostic accuracy in gynecologic cytology. Although useful for calibrating interpretive thresholds, neither allows the identification of true-positive, false-positive, true-negative, and false-negative results, all of which are necessary for calculations of sensitivity and specificity, which are prerequisites for an accuracy measurement. The accuracy of a CP can be measured after blinded review of slides or by correlation with histopathologic follow-up.

Cytology/Biopsy Correlation

Cytology/biopsy correlation provides information regarding a CP's diagnostic accuracy in a variety of ways. Data from biopsy follow-up (e.g., cervical biopsy, FNA audits) can be used to generate different statistical measures of a CP's accuracy, like the PPV. When a CP makes a positive cytologic diagnosis, how often is it confirmed by a subsequent biopsy?

The formula is:

$$\text{PPV} = \text{TP} / (\text{TP} + \text{FP}),$$

where TP is the number of cases with a positive biopsy (true-positive), and FP is the number of cases with a negative biopsy (false-positive).

Because cytologic diagnoses are not binary, but expressed in several probabilistic categories (negative, atypical, suspicious, and positive), a positive diagnosis for the purposes of biopsy correlation can be defined in several ways. For example, one can combine suspicious with positive diagnoses, but exclude atypical diagnoses. A PPV can be calculated for any specimen type, whether gynecologic or nongynecologic, but the meaning of a positive diagnosis must be defined.

A related measure is the specificity, which also takes into account FP diagnoses. The formula for specificity is

$$\text{specificity} = \text{TN} / (\text{TN} + \text{FP}),$$

where TN (true-negatives) is the number of cases called negative and confirmed as such. It is a more difficult measure to apply to CP performance evaluation because negative diagnoses are more difficult to verify.

The PPV and specificity measure only one side of accuracy—that related to false-positives (“overcalls”). FNs (“undercalls”) by the CP are equally important, and are often expressed as the sensitivity.

$$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN}).$$

Sensitivity and specificity are highly negatively correlated, meaning that improvements in specificity often come at the expense of sensitivity.⁶¹ An ideal measure of a pathologist's abilities evaluates both. In fact, sensitivity and specificity can be expressed simultaneously in a likelihood ratio (LR), the probability of a given test result if disease is present divided by the probability of the same result if disease is absent.⁶²

$$\text{LR} = \text{sensitivity} / (1 - \text{specificity}).$$

Sensitivity is sometimes called the “true-positive fraction,” and (1 - specificity) is called the “false-positive fraction.”⁶³

LRs can be expressed at different decision levels (e.g., negative, atypical, suspicious, or positive). When LR is calculated for a test at several different decision levels, the result is a receiver operating characteristic (ROC) curve. ROC curves can be generated for individual pathologists, and thresholds set for acceptable performance (Fig. 17.1).

ROC curves have been used to evaluate the accuracy of CPs interpreting breast FNAs and have shown that CPs with greater training and experience have greater accuracy.² ROC curves have shown that CPs have a better accuracy in evaluating bronchial brushings when they are provided with clinical history.⁶⁴ Finally, ROC curves

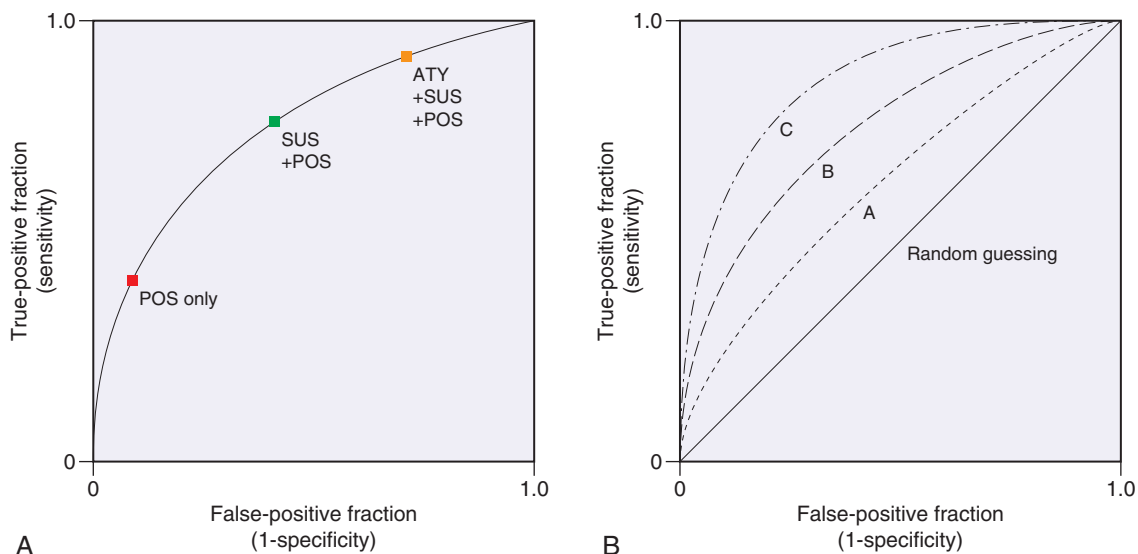


Figure 17.1 Receiver operating characteristic (ROC) curves. A, Cytologists use different decision thresholds—“atypical” (ATY), “suspicious” (SUS), “positive for malignancy” (POS)—in diagnosis. Sensitivity and specificity can be measured at each decision threshold. At the most stringent decision threshold, when only positive diagnoses are considered true positives, a point closest to the origin is generated. As the criteria become less stringent, the points move higher on the curve. B, Random guessing produces a 45-degree angle line. The accuracy of observers A, B, and C is the area under their curves. Perfect accuracy is an area of 1.0. Of the three observers, C has the best accuracy.

have been used to evaluate the accuracy of CPs reviewing Paps, where ASC, LSIL, and HSIL were treated as diagnoses of increasing stringency.⁶⁵

ROC curves, although useful for performance evaluation, have limitations, especially when applied to gynecologic cases. Biopsy data are sparse for women with negative Paps, and most negative Paps are not reviewed by a pathologist. If a PPV or ROC curve is generated from routine work, the group of Paps each pathologist reviews is different.

Finally, the biopsy is not a perfect gold standard. Note, however, that these limitations are less important if one is comparing individuals to the norm, rather than looking for absolute accuracy. For example, the imperfections of the biopsy as a gold standard are less troublesome if its imperfections affect all individuals equally. This is likely to be the case if the sample size is sufficiently large, as it is likely to be if ROC curves are measured over a 1- or 2-year period.

SAFETY

OSHA Bloodborne Pathogens Standard

The OSHA Bloodborne Pathogens Standard was published in 1991 to control the health risk associated with exposure to blood and other potentially infectious materials. Of primary concern are the human immunodeficiency virus (HIV) and the hepatitis B and C viruses, and needlestick injuries are also a major culprit. An estimated 600,000 to 800,000 needlestick and other percutaneous injuries occur every year among health care workers. In response to this public health concern, Congress passed the Needlestick Safety and Prevention Act that became law on November 6, 2000. To meet its requirements, OSHA revised its Bloodborne Pathogen Standards in 2001. The revised standard clarifies the need for employers to select safer needle devices and to involve employees in identifying and choosing these devices. The updated standard also requires employers to maintain a log of injuries from contaminated sharps.

Exposure Control

Each employer must establish a written exposure control plan.

The written exposure control plan includes:

- exposure determination
- methods of compliance
- hepatitis B vaccination and postexposure evaluation
- communication of hazards to employers
- recordkeeping

“Exposure determination” contains a list of all job classifications in which all employees have occupational exposure, and a list of all job classifications in which some employees have exposure. It also contains a list of tasks or procedures in which exposure occurs.

Methods of compliance include:

- universal precautions
- engineering and work practice controls

Because it is impossible to identify all patients whose blood may contain HIV, hepatitis B, or hepatitis C, it is advisable that blood and certain body fluids of all patients are considered to be potentially infectious. This principle of **universal precautions** was emphasized in the 1980s and is still in effect today.⁶⁶

Potentially infectious human materials include:

- blood
- semen
- vaginal secretions
- CSF
- synovial fluid
- pleural, pericardial, and peritoneal fluids
- amniotic fluid
- saliva in dental procedures
- any fluid visibly contaminated with blood
- all body fluids in situations that it is difficult or impossible to differentiate between body fluids
- any unfixed human tissue

Engineering and work practice controls include:

- handwashing facilities
- sharps disposal methods
- storage and transport methods
- personal protective equipment (PPE)
- housekeeping practices
- regulated waste removal
- safer laundry practices

Employers are encouraged to provide handwashing facilities. Employees must wash hands after removal of gloves or other PPE. If blood or other potentially infectious materials come in contact with skin or mucous membranes, the skin should be washed with soap and water, and mucous membranes flushed with water immediately.

Proper precautions are advised regarding the handling of contaminated needles and other “sharps” (scalpel blades, glass). Needles should not be bent or recapped unless no alternative is feasible, in which case the

procedure must be accomplished by mechanical device or a one-handed technique.

Contaminated sharps are discarded immediately in containers that are:

- closable
- puncture resistant
- leak-proof on sides and bottom
- labeled or color-coded
- easily accessible during use
- maintained upright
- replaced routinely and not allowed to overfill

Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in the cytopreparatory area or during an FNA procedure, in which there is a reasonable likelihood of occupational exposure.

Specimens must be placed in a leak-proof container during collection, handling, processing, storage, or transport. If outside contamination of the primary container occurs, it should be placed inside a second leak-proof container.

The employer is required to provide **PPE** to the employee. PPE includes gloves, mask, eye protection, and face shields. Gloves should be worn whenever the employee may have contact of the hands with blood or other potentially infectious materials. Masks in combination with eye protection devices (goggles or glasses with solid side shields) or chin-length face shields should be worn whenever splashing of blood or potentially infectious materials may occur and contaminate the eyes, nose, or mouth.

Contaminated work surfaces should be decontaminated with a disinfectant after completion of procedures, after surfaces are overtly contaminated, and at the end of the work shift. Protective coverings such as imperviously backed absorbent paper should be removed and replaced after they are overtly contaminated or at the end of the work shift. Broken glassware must be picked up by mechanical means (brush and dust pan) and not with the hands.

Regulated waste, such as empty specimen containers, must be placed in containers that are closable, leak proof, and labeled. Contaminated laundry must be handled as little as possible and bagged at the location it was used.

Hepatitis B Vaccination

The employer must offer free hepatitis B vaccination to all employees with potential occupational exposure, unless the employee has been previously immunized, is immune based on antibody testing, or the vaccine is contraindicated for medical reasons. Employees must either accept or sign a statement to say that they decline the vaccine. After a report of an exposure, the employer

must provide the employee with an immediate confidential medical evaluation and follow-up.

Communication of Hazards to Employees

Warning labels must be applied to containers of waste and refrigerators containing blood or other potentially infectious material. Specimen containers that are labeled as to their contents are exempted from this requirement. The labels must be fluorescent orange or orange-red, with lettering and symbols in a contrasting color (Fig. 17.2).

Employers need to ensure that all employees with occupational exposure participate in a training program, at no cost, at the time of initial assignment and at least annually thereafter.

Recordkeeping

The employer must establish and maintain a record of each employee with occupational exposure.

The record for each employee with occupational exposure must include:

- employee's name and social security number
- hepatitis B vaccination status
- post-exposure and follow-up
- written opinion of health care professional
- confidentiality

The employer must also keep a record of all training sessions attended by the employee and a sharps injury log.

OSHA Laboratory Standard

OSHA recognizes the hazards of the laboratory as a workplace and has developed a standard, often referred to as the "Laboratory Standard," for occupational exposure to hazardous chemicals. The list of hazardous chemicals in a cytology laboratory is relatively short. Nevertheless, all laboratories are required to produce



Figure 17.2 Biohazard warning label. The label is brightly colored and the lettering is easy to read.

a Chemical Hygiene Plan that specifically addresses the hazards on its premises. Numerous chemical hygiene plans from universities and health care facilities can be found on the Internet, and many are listed on OSHA's Web site.

Excluded from this standard are chemicals used in building maintenance and the production of chemicals for commercial sale; these are regulated under other OSHA standards.

The laboratory director must determine which hazardous chemicals are present in the laboratory. He or she must also monitor the exposure of employees to chemicals that might exceed the action level. OSHA provides a summary of the properties of hazardous chemicals, their health effects, and procedures for sampling the levels of exposure. **Permissible exposure limits (PELs)** have been established for hazardous chemicals. These are expressed as longer (typically 8-hour) and shorter (15-minute) time-weighted averages (TWAs). An action level is the PEL calculated as an 8-hour TWA. Exceeding it initiates required activities such as exposure monitoring.

The current OSHA PEL for xylene is 100 parts per million (ppm) for an 8-hour TWA. The odor threshold for xylene is 1 ppm. Because the odor threshold is below the current PEL of 100 ppm, xylene is considered to have adequate warning properties.

For every hazardous chemical, OSHA requires that the manufacturer develop warning labels for containers, as well as **material safety data sheets (MSDSs)**. MSDSs provide comprehensive technical information, such as PELs, and serve as a reference for exposed workers, their employer, and their health care provider. By contrast, labels provide a brief synopsis of the hazards. Training in the reading of labels and MSDSs is essential so that employees know what to wear to protect themselves and what actions are necessary in case of emergency.

All laboratories that generate hazardous waste must set up a **satellite accumulation area (SAA)**. An SAA is a designated area where the hazardous waste will be stored until it is sent out for processing. An SAA can be a bench top, a section of the hood, or an empty cabinet, but it must be near the point where waste is generated.

The satellite accumulation area must have a label with

- the words *hazardous waste*
- the chemical name in words (not formula)
- the type of hazard (e.g., ignitable, toxic)
- a place to write the date when the container is full

A designated person is called to pick up the container when it is full and arrange for its disposal. Hazardous waste cannot be poured down sinks or in waste baskets; it must be treated, disposed of in a chemical landfill, or burned in a hazardous waste incinerator approved by the Environmental Protection Agency.

National Fire Protection Association Standard for Health Care Facilities

This standard, known as NFPA 99, contains requirements for minimizing hazards, particularly as a result of fire in health care facilities. Of particular relevance to cytology laboratories, it sets the standards for the storage and use of flammable and combustible liquids in laboratories.

National Fire Protection Association Standard on Fire Protection for Laboratories Using Chemicals

This standard, known as NFPA 45, outlines the maximum allowable quantities of flammable and combustible liquids and the requirements for laboratory ventilation and hoods.

NFPA has also published a standard (NFPA 704) for recognizing and identifying specific hazards that may present as short-term exposure as a result of a fire or a spill. The system uses a diamond-shaped placard that incorporates colors and numbers. Hazard severity ranges from zero (0), which indicates a minimal hazard, to 4, the most severe hazard. The diamond is subdivided into four squares of different colors.

Each color in the diamond identifies a different hazard:

- blue: health
- red: flammability
- yellow: instability
- white: special hazards

An example is shown in Fig. 17.3. The “special hazards” are *W*, which indicates unusual reactivity with water and is a caution about using water for either fire

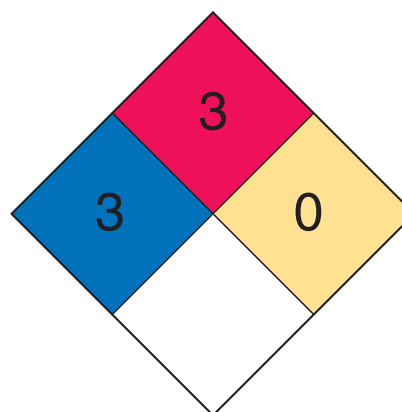


Figure 17.3 Fire safety placard. In this example, the health (blue) and fire hazards (red) are rated 3 (out of 4). The instability hazard (yellow) is zero, and there are no special hazards (white).

or spill control, and OX, which indicates that the material is an oxidizer. These placards are posted on exterior walls of a facility, any access to a room, or each principal means of access to an exterior area. It is especially important that they are visible to emergency responders. If there are many chemicals in a laboratory, professional judgment is advised to increase or decrease the severity ratings by assessing the relative quantities and their synergistic effects.

REFERENCES

- Bogdanich W: Lax laboratories: the Pap test misses much cervical cancer through labs' errors. *Wall Street Journal* 1987;210(88):1,20.
- Clinical Laboratory Improvement Amendments of 1988: Final Rule. Fed Reg: United States Department of Health and Human Services, United States Government Printing Office 2003;3710-3711.
- Ashton P: Clinical laboratory surveys. ASCT Cytopathology Quality Assurance Guide, 2nd ed., vol. I. Raleigh, American Society for Cytotechnology, 2001.
- Triol JH, Russell CD, Ashton PR: Health Care Financing Administration/American Society for Cytotechnology inspections: Government assessment of cytology laboratory practice under the regulations of the Clinical Laboratory Improvement Amendments of 1988. *Arch Pathol Lab Med* 1997;121:264-266.
- www.cms.hhs.gov/CLIA/downloads/CLIA092006CytologyUpdate.pdf.
- Clinical and Laboratory Standards Institute (CLSI): Laboratory Documents: Development and Control; Approved Guideline, 5th ed. CLSI document GP2-A5 Wayne, Pennsylvania, 2006.
- Triol JH, Goodell RM: ASCT Cytopathology Quality Assurance Guide. 2nd ed. Raleigh, American Society for Cytotechnology, 2001.
- Voytek TM, Mody DR, Davey DD: Quality assessment and improvement in cytopathology. In Nakhleh RE, Fitzgibbons PL (eds): *Quality Improvement Manual in Anatomic Pathology*, 2nd ed. Northfield, IL, College of American Pathologists, 2002, pp.55-96.
- Padgett DL: *Pathology Service Coding Handbook*, version 7.3. Simpsonville, KT, DL Padgett Enterprises, Inc., 2007.
- American Medical Association: *Current Procedural Terminology (CPT®) 2008*. Chicago, AMA Press, 2008.
- 2008 Hospital and Payer Professional ICD-9-CM International Classification of Diseases, Volumes 1, 2, and 3. Salt Lake City, The Medical Management Institute, 2007.
- Hutchinson ML, Isenstein LM, Goodman A, et al.: Homogeneous sampling accounts for the increased diagnostic accuracy using ThinPrep Processor. *Am J Clin Pathol* 1994;101:215-219.
- Clinical Laboratory Improvement Amendments of 1988, Final Rule: Fed Reg: United States Department of Health and Human Services, United States Government Printing Office 1992, 7001-7288.
- Melamed MR: Quality control in cytology laboratories. *Gynecol Oncol* 1981;12:S206-S211.
- Inhorn SL, Shalkham JE, Mueller GB: Quality assurance programs meet CLIA requirements. *Diagn Cytopathol* 1994;11:105-200.
- Koss LG: Cervical (Pap) smear: new directions. *Cancer* 1993;71:1406-1412.
- Rohr LR: Quality assurance in gynecologic cytology: What is practical? *Am J Clin Pathol* 1990;94:754-758.
- Tabbara SO, Sidawy MK: Evaluation of the 10% rescreen of negative gynecologic smears as a quality assurance measure. *Diagn Cytopathol* 1996;14:84-86.
- Allen KA, Zaleski S, Cohen MB: Review of negative Papanicolaou tests: Is the retrospective 5-year review necessary? *Am J Clin Pathol* 1994;101:19-21.
- Gatscha RM, Warren GP, Saigo PE: Quality assurance: Insight into a laboratory's performance [Abstract]. *Lab Med* 1994;25:258.
- Hatem F, Wilbur DC: High grade squamous cervical lesions following negative Papanicolaou smears: False-negative cervical cytology or rapid progression. *Diagn Cytopathol* 1995;12:135-141.
- Jones BA: Rescreening gynecologic cytology: Rescreening 3762 previous cases for current high-grade squamous intraepithelial lesions and carcinoma—a College of American Pathologists Q-Probe study of 312 institutions. *Arch Pathol Lab Med* 1995;119:1097-1103.
- Montes MA, Cibas ES, DiNisco SA, Lee KR: Cytologic characteristics of abnormal cells in prior "normal" cervical/vaginal Papanicolaou smears from women with a high grade squamous intraepithelial lesion. *Cancer (Cancer Cytopathol)* 1999;87:56-59.
- Nick TG, Bush DE, Cason Z, Lemos LB: Review of negative Pap smears: Quality assurance protocol for high-grade squamous intraepithelial lesions [Abstract]. *Acta Cytol* 1995;39:990.
- Tabbara SO, Sidawy MK: Evaluation of the 5-year review of negative cervical smears in patients with high grade squamous intraepithelial lesions. *Diagn Cytopathol* 1996;15:7-11.
- van der Graaf Y, Vooijs GP, Gaillard HL, Go DM: Screening errors in cervical cytologic screening. *Acta Cytol* 1987;31:434-438.
- Dodd LG, Sneige N, Villareal Y, et al.: Quality-assurance study of simultaneously sampled, non-correlating cervical cytology and biopsies. *Diagn Cytopathol* 1993;9:138-144.
- Ibrahim SN, Krigman HR, Coogan AC, et al.: Prospective correlation of cervicovaginal cytologic and histologic specimens. *Am J Clin Pathol* 1996;106:319-324.
- Joseph MG, Cragg F, Wright VC, et al.: Cyto-histological correlates in a colposcopic clinic: A 1-year prospective study. *Diagn Cytopathol* 1991;7:477-481.
- Joste NE, Crum CP, Cibas ES: Cytologic/histologic correlation for quality control in cervicovaginal cytology: Experience with 1,582 paired cases. *Am J Clin Pathol* 1995;103:32-34.
- Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol* 2003;188(6):1383-1392.
- A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. *Am J Obstet Gynecol* 2003;188(6):1393-1400.
- Jones BA, Novis DA: Cervical biopsy-cytology correlation. A College of American Pathologists Q-Probes study of 22,439 correlations in 348 laboratories. *Arch Pathol Lab Med* 1996;120:523-531.
- Solomon D, Schiffman M, Tarone R: Comparison of three management strategies for patients with atypical squamous cells of undetermined significance; baseline results from a randomized trial. *J Natl Cancer Inst* 2001;93:293-299.
- Chute DJ, Covell J, Pambuccian SE, Stelow EB: Cytologic-histologic correlation of screening and diagnostic Papanicolaou tests. *Diagn Cytopathol* 2006;34(7):503-506.
- Collins LC, Wang HH, Abu-Jawdeh GM: Qualifiers of atypical squamous cells of undetermined significance help in patient management. *Mod Pathol* 1996;9:677-681.
- Hall S, Wu TC, Soudi N, Sherman ME: Low-grade squamous intraepithelial lesions: Cytologic predictors of biopsy confirmation. *Diagn Cytopathol* 1994;10:3-9.
- Tabbara S, Saleh AM, Barber S, et al.: The Bethesda classification for squamous intraepithelial lesions: Histologic, cytologic and viral correlates. *Obstet Gynecol* 1992;79:338-346.

39. Brown FM, Faquin WC, Sun D, et al.: LSIL biopsies after HSIL smears: Correlation with high-risk HPV and greater risk of HSIL on follow-up. *Am J Clin Pathol* 1999;112:765-768.
40. Tritz DM, Weeks JA, Spires SE, et al.: Etiologies for non-correlating cervical cytologies and biopsies. *Am J Clin Pathol* 1995;103:594-597.
41. Krieger P, Naryshkin S: Random rescreeing of cytologic smears: A practical and effective component of quality assurance programs in both large and small cytology laboratories. *Acta Cytol* 1994;38:291-298.
42. Nagy GK, Collins DN, Wilson TA: Sample size calculations for rescreeing cytologic smears. *Acta Cytol* 1996;40:501-505.
43. Sherman ME, Schiffman MH, Lorincz AT, et al.: Toward objective quality assurance in cervical cytopathology: Correlation of cytopathologic diagnoses with detection of high-risk human papillomavirus types. *Am J Clin Pathol* 1994;102:182-187.
44. Young NA, Naryshkin S, Atkinson BE, et al.: Interobserver variability of cervical smears with squamous-cell abnormalities: A Philadelphia study. *Diagn Cytopathol* 1994;11:352-357.
45. Stoler MH, Schiffman M: Interobserver reproducibility of cervical cytologic and histologic interpretations: Realistic estimates from the ASCUS-LSIL triage study. *JAMA* 2001;285:1500-1505.
46. Krieger PA, Cohen T, Naryshkin S: A practical guide to Papanicolaou smear rescreens: How many slides must be re-evaluated to make a statistically valid assessment of screening performance? *Cancer (Cancer Cytopathol)* 1998;84:130-137.
47. Naryshkin S: The false-negative fraction for Papanicolaou smears: How often are "abnormal" smears not detected by a "standard" screening cytologist? *Arch Pathol Lab Med* 1997;121:270-272.
48. Lo JW, Fung CHK: Simple BASIC program for calculating the cervicovaginal FNP and for estimating the sample size of the number of cervicovaginal smears to be rescreened. *Acta Cytol* 1999;43:999-1005.
49. Renshaw AA, DiNisco SA, Minter LJ, Cibas ES: A more accurate measure of the false-negative rate of Papanicolaou smear screening is obtained by determining the false-negative rate of the rescreeing process. *Cancer (Cancer Cytopathol)* 1997;81:272-276.
50. Pagano M, Gauvreau K: *Principles of Biostatistics*, 2nd ed. Pacific Grove, CA, Duxbury Press, 2000.
51. Cibas ES, Dean B, Maffeo N, Allred EN: Quality assurance in gynecologic cytology; the value of cytotechnologist-cytopathologist discrepancy logs. *Am J Clin Pathol* 2001;115:512-516.
52. Fleiss JL: *Statistical Methods for Rates and Proportions*, 2nd ed. New York, John Wiley, 1988.
53. Kurman RJ, Luff R, Solomon D, Henson D: *The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses: Definitions, Criteria, and Explanatory Notes for Terminology and Specimen Adequacy*. New York, Springer-Verlag, 1994.
54. Juskevicius R, Zou KH, Cibas ES: An analysis of factors that influence the ASCUS/SIL ratio of pathologists. *Am J Clin Pathol* 2001;116:331-335.
55. Nascimento AF, Cibas ES: The ASC/SIL ratio for cytopathologists as a quality control measure: A follow-up study. *Am J Clin Pathol* 2007;128(4):653-656.
56. Stoler MH: ASC, TBS, and the power of ALTS. *Am J Clin Pathol* 2007;127(4):489-491.
57. Chhieng DC, Chen J, Connolly K, et al.: High-risk HPV DNA detection rate in patients with atypical squamous cells and its relationship to the atypical squamous cell: Squamous intraepithelial lesion ratio. *Acta Cytol* 2006;50(3):291-294.
58. Zuna RE, Moore W, Dunn ST: HPV DNA testing of the residual sample of liquid-based Pap test: Utility as a quality assurance monitor. *Mod Pathol* 2001;14(3):147-151.
59. Cibas ES, Zou KH, Crum CP, Kuo F: Using the rate of positive high-risk HPV test results for ASC-US together with the ASC-US/SIL ratio in evaluating the performance of cytopathologists. *Am J Clin Pathol* 2008;129(1):97-101.
60. Solomon D, Schiffman M, Tarone R: Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *JNCI* 2001;93:293-299.
61. Fahey MT, Irwig L, Macaskill P: Meta-analysis of Pap test accuracy. *Am J Epidemiol* 1995;141:680-689.
62. Raab S: Diagnostic accuracy in cytopathology. *Diagn Cytopathol* 1994;10:68-75.
63. NCCLS: *Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots; Approved Guideline*. NCCLS Document GP10-A (ISBN 1-56238-285-3). Wayne, PA, NCCLS, 1995.
64. Raab SS, Thomas PA, Lenel JC, et al.: Pathology and probability: Likelihood ratios and receiver operating characteristic curves in the interpretation of bronchial brush specimens. *Am J Clin Pathol* 1995;103:588-593.
65. Renshaw AA, Dean BR, Cibas ES: Receiver operating characteristic curves for analysis of the results of cervicovaginal smears: A useful quality improvement tool. *Arch Pathol Lab Med* 1997;121:968-975.
66. Centers for Disease Control: Recommendations for prevention of HIV transmission in health-care settings. *MMWR* 1987;36(Suppl. 25):1S-18S.

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